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REVIEW AND EVALUATION OF AVAILABLE TECHNIQUES FOR DETERMINING PERSISTENCE AND ROUTES OF DEGRADATION OF CHEMICAL SUBSTANCES IN THE ENVIRONMENT

SYRACUSE UNIVERSITY RESEARCH CORPORATION

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16. ABSTRACT
This report reviews and evaluates the present state of techniques which have been used to determine the environmental persistence (biological, chemical and photochemical degradation) and routes of degradation of chemicals released in the environment by human activities. The information sources included relevant papers, books, and review articles, abstracting services, and computer searches such as National Technical Information Services, and the current investigators files of the Smithsonian Science Information Exchange.

The techniques that were identified were reviewed and then evaluated for their ability to simulate natural environmental conditions, convenience of procedure, time requirements, necessary equipment and reproducibility. The ultimate evaluation was based on how well the methods have worked with well-known environmental contaminants. A cost analysis of the test methods was undertaken to determine the feasibility of comprehensive screening of chemicals for environmental persistence.

A relationship between chemical structure and environmental persistence is presented and some theoretical grounds for such correlations are discussed. An attempt has been made to categorize chemicals for their suitability to various test methods based on consideration of physiochemical properties, toxicity, environmental release factors and commercial economic factors.

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SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

The determination of persistence and degradation products of a chemical contaminant in the environment is an important parameter in the overall evaluation of a compound's potential environmental hazard. This report reviews and evaluates techniques which have been used to study the persistence and degradation metabolites of chemicals in the environment.

A literature search was conducted using papers, books, and review articles, abstracting services, and computer searches such as the National Technical Information Service and the current investigator files of the Smithsonian Science Information Exchange. Relevant articles were gathered and examined, and approximately 800 references were used in the review and evaluation report.

A major difficulty encountered in this review is the lack of precise definitions or criteria for evaluating a chemical substance's stability in the environment. Such terms as environmental persistence, alteration, and biodegradability have rarely been assigned quantitative definitions. This is due to the numerous environmental factors which affect the degradation rate and the lack of precision inherent in characterizing biological systems. Thus, even if one microenvironment is exactly simulated in the laboratory, application of the results to other environments can be at best only quantitative. This is in contrast to the half-life of an isotope, for example, where the rate is independent of environmental systems.

Several definitions have been suggested, especially for biodegradability:

Primary biodegradation - biodegradation to the minimum extent necessary to change the identity of the compound (WPCF, 1967)

Ultimate biodegradation - biodegradation to (a) water, (b) carbon dioxide, (c) inorganic compounds (WPCF, 1967)

Acceptable biodegradation - biodegradation to the minimum extent necessary to remove some undesirable property of the compound such as foaminess or toxicity (WPCF, 1967)

Very biodegradable - a compound that can be utilized as a carbon and energy sources (Prochazka and Payne, 1965)

Biodegradable - a compound that can be attacked by the enzyme apparatus acquired by microbes during the course of evolution (Dagley, 1972b)

In addition, some researchers have defined biodegradability in terms of specific analytical methods and test systems. For example, the presumptive test of the Soap and Detergent Association (SDA, 1965) defines as biodegradable any anionic surfactant that loses greater than 90% of its methylene blue activated substances (MBAS) in the SDA shake culture test. These types of definitions are useful for comparison of different compounds in closely related chemical groups. Furthermore, there is considerable dispute over whether a compound that cannot be used for growth may be cometabolized (concomitant metabolism of a non-growth substrate) to a significant extent in the natural environment. All of these definitions have some merit and drawbacks. We would suggest that a truely biodegradable compound is one which can be converted in a relatively short period of time to low-molecular-weight metabolic intermediates (e.g. glutamic acid, succinic acid, etc.).

The qualitative nature of these definitions is apparent and it is probable that these definitions will remain relatively inexact because of the variety of chemical compounds being considered.

A chemical that is released into the environment may be affected by physical transport processes or altered chemically by exposure to various biological and chemical agents in the environment. The physical processes have a tendency to distribute and dilute or concentrate but not destroy the contaminant and these transport processes can have considerable impact on the biological and chemical reactions that may take place. On the other hand, chemical and biological reactions in the environment result in alterations and frequently degradations of the contaminants to substances which in many cases may be innocuous, thus ending the pollution potential of the contaminant.

The alteration and degradation processes can be divided into three categories: (1) biodegradation - effected by living organisms, (2) photochemical degradation - nonmetabolic degradation requiring light energy, and (3) degradation by chemical agents (chemical degradation) - nonmetabolic degradation which does not require sunlight. Biodegradation of organic compounds appears to be the most desirable because it results generally in completely mineralized end-products. In contrast, photochemical and other nonmetabolic processes usually result in only slight modifications in the parent compound. Microorganisms appear to have major responsibility for determining persistence or breakdown of an environmental chemical and, therefore, techniques which use microorganisms have been focused upon in this review. Many compounds that enter the environment (e.g. pesticides and hydrocarbons) have been shown to be photochemically labile, but the importance of the process for soil and water contaminants is unknown. Hydrolysis of environmental compounds by chemical agents has been extensively studied and correlation between laboratory and field results is facilitated by the ease of measuring one of the more important rate-determining factors, pH, both in the laboratory and in the field. However, other nonmetabolic processes have received little attention.

A variety of techniques have been used to determine biodegradation in soil and water. They are briefly tabulated below and advantages and disadvantages are outlined in the subsequent paragraphs. Following those paragraphs are discussions of techniques used to study photochemical and other nonmetabolic processes, metals, and polymeric materials. Then conclusions and recommendations are presented.

Water

- Biochemical Oxygen Demand (BOD)
  - Dilution Method
  - Respirometry
- River Die-Away
- Shake Culture Test
  - Mixed Cultures
  - Pure Cultures
- Model Ecosystems
- Activated Sludge
  - Official German Test
  - Miniature Continuous
  - Semicontinuous
- Trickling Filter
- Anaerobic Systems

Soil

- Laboratory Techniques
  - Natural and Mixed Cultures
    - Soils Incubated with Test Chemical
    - Soils Suspended in Aqueous Solution
    - Soil Perfusion
  - Pure Cultures
  - Cell Free Extracts
- Greenhouse Studies
- Field Studies

Many of the above techniques are generally considered suitable for rapid screening of biodegradability, whereas the others have been more commonly used for detailed investigation of the biodegradation process. With all the techniques used, the analytical method provides the greatest spectrum of cost and desirable information. Using radiolabelled compounds is undoubtedly the best way to study a chemical's persistence and breakdown, but the cost of synthesizing the compound is frequently high. At the other end of the spectrum are techniques which require no analytical development work (e.g., BOD, CO_2 evolution and total organic carbon). In between are techniques such as thin layer and gas chromatography.

Rapid Screening Tests for Biodegradation in Water

Prior to an extensive investigation of the pathways of biodegradation of chemical substances, a rapid screening test is usually run. Perhaps the most frequently run test is the determination of biochemical oxygen demand either by the dilution method or respirometry. Since the method requires no specific analytical technique for estimation of the chemical compound, the test is rapid and can be applied to a variety of chemical compounds. Further, since the procedure measures oxygen consumption and not the compound, there can be little confusion about disappearance of the compound due to physical adsorption of the test chemical. The major difficulty with this technique is quite often the interpretation of the results. The method does not provide any information about the nature of the degradation products. Also, since oxygen is utilized for a number of complex metabolic reactions (including synthesis of cell material) and not simply for oxidation of the test chemical, the correlation of oxygen utilization data to loss of test compound to assess the extent of biodegradation is some times difficult unless extremes of oxygen uptake are noted (0% or 100% of theoretical O_2). In addition, the endogenous oxygen uptake rate is usually subtracted from the oxygen uptake measured in the presence of the test chemical, ignoring the possibility of stimulation or inhibition of the endogenous rate sometimes caused by addition of the test chemical. Simultaneous measurement of soluble carbon during disappearance of the test compound may be used to confirm and supplement the respirometric data. The respirometric method is generally considered more precise as far as measurement of oxygen demand is concerned, and since the microbial concentrations used in the respirometric method are usually high, the technique simulates treatment plant conditions more closely than does the dilution method. The polarographic method of respiration measurement is generally less time consuming and allows continuous monitoring of oxygen demand (BOD is normally only measured after 5 days) but since the reaction period is so short little acclimation is allowed.

The river die-away test is relatively simple and requires a minimum of equipment. The test chemical is placed in a natural water sample and the disappearance of the compound is monitored. The reaction conditions closely approximate the conditions encountered in nature. However, one of the more serious shortcomings of the test is the variations in bacterial count and composition between different rivers, between different points in the same river, and even at the same point in a given river, which may cause considerable fluctuation in the results.

Shake culture systems inoculated with natural communities of microorganisms are also used as a screening test, the most standardized being the SDA (1965) method. The source of microbial inoculum has included such sources as sewage (most frequent), lagoons, and lake sediments, but the well defined medium composition which is used, provides somewhat better reproducibility than the river die-away test. The technique allows for flexible operation including the use of acclimated and unacclimated seed, a defined external carbon source, and even a preserved seed (better reproducibility, but less like a natural population). Generally high concentrations of microorganisms are used. As a result the test period is relatively short but the test conditions may be more favorable for degradation than generally encountered in the natural environment.

To date, model ecosystems are oriented more towards answering questions of bioaccumulation and metabolism of chemical substances in upper levels of the food chain. Although metabolism in higher food chain organisms is important in terms of toxicological effects and bioaccumulation, its importance in terms of total environmental persistence or degradation appears to be relatively minor in comparison to the role of microorganisms. Thus it appears to make little sense to use elaborate model ecosystems which are time consuming and difficult to set up to test for environmental persistence and biodegradability. However, it should be noted that many of the results obtained from model ecosystems for highly degradable or very persistent chemicals seem to be in good agreement with environmental monitoring data and microbial test systems. However a striking exception is benzoic acid which is very biodegradable in microbial systems but in the system of Metcalf *et al.* (1971) the compound is accumulated and conjugated in the food chain organisms.

Test Methods for Determining Biodegradability in Biological Waste Water Treatment Plants

Techniques used to simulate biological waste water treatment plants differ in microbial population and concentration and frequently in the amount of acclimation that is developed with the test compound. Since numerous chemicals which potentially could enter the environment first pass through biological waste water treatment plants, it is important to determine their fate in these systems.

Activated sludge systems are most frequently modelled in the laboratory. Both continuous and semicontinuous procedures are used. The semicontinuous units are much more economical since they do not require the constant attention of a continuous system and they use much less feed and test material. Also, semicontinuous operation avoids the difficulty of maintaining satisfactory circulation of sludge which is sometimes encountered using continuous systems.

The official German activated sludge test has been required by German law since 1964 for testing anionic surfactants. The technique has the advantage that it can be run in any well equipped laboratory (no sewage effluent or sludge needed as inoculum because the sludge is developed from airborne microorganisms). Disadvantages include the fact that several researchers have found it difficult to maintain a stable biochemical operation and satisfactory circulation of sludge. The latter problem seems to be remedied by the porous pot modification.

The miniature continuous-flow activated sludge systems provide economy in preparation, storage and handling of feeds as well as savings in time and labor. Although miniature systems are further removed from the characteristics of full scale treatment plants, the large scale units are so far removed from full scale that another factor of 10 probably has little affect. Both natural and synthetic feed have been used, the natural feed providing shorter acclimation time but requiring that radiolabelled material be used with surfactants because of the background of surfactants in the natural feed.

Although the semicontinuous technique does not exactly simulate the continuous operation of a full treatment plant, it is similar to plants where the feed and recycled sludge are mixed at the entrance to the aerator. The 24 hour cycle of the SDA procedure is convenient because it requires no overnight attention.

Trickling filters are somewhat easier to operate than continuous activated sludge systems, since no sludge needs to be recycled. Also, scale-up factors from laboratory studies to commercial filters can be made without great worry since the most important dimension is the depth of the bed. However, disadvantages include: (1) a long acclimation period (14 weeks for development of a mature film and 4-8 weeks acclimation), (2) a fly nuisance, (3) lack of easy accommodation in a constant-temperature room or bath, and (4) operational conditions can not be readily adjusted (especially retention time). Recirculating filter tests, which are very similar to soil perfusion tests (except that the supplemental nutrients and carbon are higher in the recirculating filter), seem to be of less value because their high biodegradability potential does not allow distinction between relative degrees of biodegradability.

Soil Screening Tests

Unlike natural water, soils are generally rich in microorganisms and, therefore, have been used extensively as microbial inoculum without amendment or added microorganisms. Test methods utilizing natural communities of microorganisms have generally been used as screening tests for biodegradability.

Common techniques include soil perfusion and incubating of the test chemical with soil or with soil suspended in water. Using the soil as the test medium closely simulates the conditions encountered in nature. However, soil from different geographic locations may give different results in degradation studies. The presence of a complex medium such as soil may introduce additional steps in extraction and clean up and some metabolites may be unextractable. Also, the presence of soil may preclude the assay of biodegradation by many analytical procedures (e.g., oxygen consumption perhaps due to the high endogenous rates of soil respiration).

In degradation test methods where soil is suspended in water, one deals with a suspension of soil, rather than soil as it is encountered under natural conditions. Since soil is used as an inoculum in the test procedure and not as a medium for degradation, the quantity of soil suspended in the aqueous medium is very small, and this may limit the availability of many undefined nutrients present in soil and considerably affect the test results. The advantage of the method is that in cases where a dilute soil suspension is used, certain analytical measurements (e.g., disappearance of U.V. absorption) can be made directly on the sample or on the supernatant obtained after centrifugation. The extraction and clean up steps in such cases will be minimal. Thus if a large number of compounds are to be tested, this technique may be contemplated.

Soil perfusion systems consist of soil columns through which a continuous flow of water passes. The test compound may be either adsorbed on the soil or dissolved in the water. Similar to aqueous suspensions, soil perfusion systems deal with a solution rather than just soil and the constant exposure of microorganisms to air, water and the test chemical may poorly simulate what occurs under natural conditions. The system is very potent and provides an unusually high biodegradability potential. Thus it appears that although the soil perfusion system is an excellent tool for enriching microorganisms which will degrade the test chemical, such a system is unsuitable for use as a routine biodegradation test method due to its unusually high biodegradation potential and difficulties in handling many units.

Enrichment and Pure Cultures

Mixed culture tests are more frequently used as screening tests because mixed cultures of microorganisms are easier to obtain and the results may be extrapolated to natural conditions easily. However, in some cases enriched or stock cultures have been used to determine biodegradability. The procedure with stock cultures usually involves screening a large number of different organisms obtained either commercially or isolated from natural sources (without enrichment) to determine the number that will grow on the test chemical. The number of different organisms that grow on a chemical can be considered somewhat indicative of the compound's biodegradability. Pure culture studies, however, fail to account for breakdown of a compound by synergistic processes and to consider the relative population density of the particular organisms as it naturally occurs in the environment.

Enrichment culture techniques usually consist of isolation of cultures which have first been enriched on the test chemical as the sole source of carbon. The result is a pure culture that is able to use the test compound as a sole source of carbon and energy. This is similar to the result from screening large numbers of pure cultures, except that the enrichment procedure is normally less time consuming. Enrichment culture using an external carbon source (chemical analogue of the test chemical - analogue enrichment; unrelated chemical - co-substrate enrichment) allows for the isolation of organisms which may cometabolize the test compound. This procedure is particularly important for compounds which have been found to be recalcitrant to being used as a carbon and energy source.

Techniques for Determining the Routes of Biodegradation

Elucidation of the pathways of degradation involves identification of the degradative intermediates and assignment of places in the scheme of degradation. However, from the point of view of understanding the environmental behavior and hazards of a chemical, it may be sufficient to identify the degradation intermediates only.

The isolation and identification of metabolites using the complete incubation mixtures from the above-mentioned screening methods is generally very difficult. The researcher is dependent upon available extraction techniques and on his knowledge of the type of breakdown products that might be expected. Also, in a mixed culture laboratory system a metabolite may be degraded rapidly and not accumulate in detectable levels. To overcome these problems, researchers have frequently used pure cultures and cell-free extracts in metabolic studies.

Major advantages of using pure cultures or cell-free extracts are (1) complications originating from the complexities and variability of the soil and water systems are eliminated, (2) the extraction and clean up procedures will be simpler, (3) data will be more reproducible, and (4) it is possible to study individual degradation steps because of the specificity of the enzyme systems contained in different species. The disadvantages include (1) failure to allow breakdown by synergistic processes and (2) the general remoteness of the conditions from natural processes in soil or water.

Cell-free extracts are normally used where intact cells provide (1) difficulty in manipulation of physical and chemical parameters, (2) rapid reactions that do not allow examination of intermediate metabolites, and (3) difficulty in studying the separate enzymes involved in degradation. Major disadvantages in the use of cell-free extracts are that (1) they are further removed from natural conditions because the permeability barrier (cell membrane) is absent and (2) the intermediates that are detected may never appear outside the cell.

Catabolic pathways are generally determined by using radiolabelled material. This is because of the higher sensitivity of radiotracer detection, and the fact that radiotracer studies provide total balance sheets of the fate of the compound. The techniques used for studying biodegradation are generally similar to those which have been used by microbial physiologists for studying metabolic pathways of a natural substance. Metabolic information with intact cells has been derived using a number of techniques, including (1) analysis of culture fluid for metabolites (2) removal of the intermediates from the cell by extraction (3) accumulation of an intermediate by addition of an enzyme inhibitor (4) sequential enzyme induction by the substrate to enable cells to oxidize intermediate oxidation product. When cell-free extracts are used, efforts are usually made to break the reaction link at different points and thus study the reaction in small segments.

This is generally accomplished by inactivation or removal of a particular enzyme. Some information on metabolic pathways can also be obtained by demonstration of the presence of appropriate enzymes in the cell-free extracts. It is clear from the above that for elucidation of metabolic pathways, there is no single method which will provide all the needed information; the researcher generally has to try to put together a pathway from the information which is derived from all the studies. For this reason, detailed studies are quite time consuming (and costly) and require a well equipped laboratory.

Photochemical Techniques

Photochemical techniques can be divided into two categories (1) simulations of atmospheric phenomena, and (2) simulations of other environmental photochemical processes.

Atmospheric studies are usually conducted on relatively volatile compounds and techniques that have been used include (1) long-path infrared (LPIR) cell reactors, (2) plastic bag reactors, (3) glass flask reactors, and (4) smog chambers. The system has the advantage of requiring minimum analytical development since most compounds have diagnostic infrared absorptions. However, because of the nature of the analytical system, relatively high concentrations and simple reaction systems must be used. Use of plastic containers and glass reactors provides inexpensive and versatile methods of studying atmospheric degradation. However, the development of analytical techniques may be quite time consuming, especially because of the small sample size. Smog chambers have the advantage of a large size (lower surface/volume and, therefore, less wall reactions; larger analytical samples and thus lower concentrations of reactants) and the potential for dynamic operation (closer to natural conditions). In addition, it is easier to control humidity and temperature in a smog chamber, but cleaning is more difficult than with plastic or glass reactors. Good correleation between laboratory and field results has been demonstrated for all systems, perhaps because of the better reproducibility of chemical systems. Few low volatile compounds have been studied under atmospheric conditions, although this may be a major reaction media for photolysis in the environment.

Photochemical processes in other parts (e.g., soil and water) of the environment are poorly understood. A number of well known environmental contaminants (e.g. pesticides) have been shown to be photochemically labile under laboratory conditions. However, the relative importance of the process to over-all environmental degradation is poorly demonstrated. Laboratory techniques have included photolyzing aqueous and organic solvent solutions, thin films, and absorbed films of the test compound both with and without photosensitizers. However, extrapolation of the laboratory results to the field is extremely difficult because of the lack of understanding of the effect that adsorption on soil or sediment or exposure to natural chemicals in the environment may have on the photochemical process. Complicating the problem is the fact that frequently the photooxidative product is the same as the product obtained microbially. It is suggested that these studies have somewhat lower priority for routine testing than do biological or chemical processes because of the difficulty inherent in interpreting the results. When a compound is tested, light containing wavelengths no less than 290 nm and a variety of test media such as water (various pH's), soil, silica gel, and thin films on glass should be used. Also, the possibility of sensitized photochemical alterations should be investigated.

Techniques for Studying Degradation Induced by Chemical Agents

Environmental processes effected by chemical agents are usually studied by eliminating the possibility of biological degradation through sterilization or using distilled water. The most frequently studied chemical process, hydrolysis, is normally determined with distilled water at various pH's. The number of compounds that should undergo this type of testing can be significantly reduced by considering the chemical structure of the compound. Esters, amides, and carbamates are obvious candidates for testing. Compounds that have good leaving groups (e.g. halogens) located at positions that would stabilize a carbonium ion (allylic, benzylic, etc.) also should be tested. Correlation of results from the laboratory to the field is facilitated by the ease of measuring in both places the pH, which has a major impact on the reaction rate.

Chemical processes in soil can be studied after sterilization of the soil by autoclaving, chemical treatment or γ-irradiation. The sterilization processes often alter the soil to such an extent that any process observed could be artificial. Nevertheless, it should be noted that any biodegradation technique where the test chemical is incubated with a natural medium (e.g. soil or water) would allow transformations effected by chemical agents to take place, and, therefore, these processes would be partially considered during biodegradation testing.

Techniques for Studying Metal Transformations

The study of metals and metal compounds transformations in the environment requires a variety of different considerations than for organic chemicals, since the metal portion of the compound can not be converted to innocuous end products, as with the organics (CO_2, H_2O). Also, some of the reactions are reversible and thus the kinetics become very important to the cycling of the metal through the environment. A variety of processes may take place such as degradation of organometallic forms to inorganic forms and valence changes, methylation, or chelation of the metal. As with organic compounds, natural communities and pure cultures and model ecosystems have been used. However, in most cases these techniques only answer the qualitative aspects of metal transformations (e.g. will a metal be methylated). Qualitative information is important for organometallic compounds and organic compounds, since the processes are irreversible. However, with metals, methylation and demethylation can both take place so that the reaction rates are extremely important. These reaction rates will vary from test method to test method and within various environments. Thus, although laboratory techniques are important for determining organometallic degradation and the possibility of metal methylation, the field studies should be used to determine the kinetics of metal transformations.

Techniques for Studying Polymeric Materials

Polymeric materials, especially plastics, are major environmental contaminants. However, the tests used with these materials have been oriented at determining deterioration (loss of commercial function) rather than degradation. This is reflected in the test methods (screening, end use, field test) and the analytical methods used (e.g. hardness, embrittlement, electrical properties). Nevertheless, the screening, end use, and field tests used all indicate that most of the synthetic organic polymers are extremely persistent, and this is supported by monitoring data.

The apparent recalcitrance to microbial attack of most commercially important synthetic polymers may well reflect physical nonavailability of the polymer to appropriate extracellular enzymes. This is to say that most synthetic polymers cannot be assimilated by microorganisms without prior chain cleavage either by exo-enzymes or physiochemical processes. While standard test methods do expose the polymers to exo-enzymes and selected physiochemical factors such as light and water, the inter-relationships between biological and physiochemical degradation are only beginning to be explored. Further, the effects of a typical microenvironments is little understood. Lastly, the environmental degradation of some polymers may be an extremely slow but nonetheless significant process and one which most exposure and analytical methods would fail to detect. Thus, more varied and prolonged exposure conditions and perhaps the use of radiolabelled material may be required to determine with confidence the environmental fate of polymers.

Conclusions and Recommendations

Review of the techniques available for studying environment persistence and pathways of degradation provides no single technique that is quick, reasonably priced, and provides information meaningful to a large number of processes in nature. In fact, none of the methods provide results that can be more than qualitatively relied upon in terms of persistance of the test chemical in nature because of the varying conditions encountered in the environment. A good method would be to radiolabel the compound and then test for its persistence and identify the breakdown products in a variety of simulated environments (not model ecosystems). This certainly should be undertaken with chemicals that are toxic and/or are reaching the environment in large quantities.

However, with chemicals that are produced in small quantities or have just reached commercial production, the justification of such extensive testing is questionable, unless the compounds exhibit a high level of toxicity. Thus, it appears that the amount and type of testing to be recommended should be decided on a case by case basis.

A number of parameters can assist in the decision making process. The quantity produced and released to the environment, the persistence, and the toxicity of the material provide some indication of the environmental hazard involved and thus the degree of testing needed. Routes into and residence in the environment provide information on the type of medium that should be used (sewage, natural water, soil, air). If the release is in only a few places it might be worthwhile considering a test that closely simulates those places (e.g. river die-away test). On the other hand, when the release is not well understood or if the compound is expected to be widely dispersed, it might be advisable to use more standardized and, therefore, somewhat more reproducible techniques. Physical properties may be useful in deciding the type of testing and the experimental procedure. For example, non-volatile compounds should not be tested in atmospheric systems and water insoluble compounds require special experimental precautions to prevent adsorptive losses. Chemical structure may also be helpful in setting priorities as well as in estimating persistence and breakdown pathways. This has been reviewed in detail in Section VIII., p. 461.

During this review a number of areas were identified where research is needed. Some of these areas are listed below:

Biomass and Metabolic Rate Considerations - Although it would appear that microorganisms determine the persistence or degradation of a chemical contaminant, much better biomass and metabolic rate information is necessary to compare microorganisms with higher organisms.

Qualitative and Quantitative Comparisons Between Laboratory Techniques and the Field - The lack of quantitative comparisons is distressing and is normally attributable to a lack of planning for physical transport processes in field monitoring research. Also, considerably more qualitative comparisons are necessary.

Field Tests to Evaluate Photochemical Processes - Although a number of chemicals have been found to be labile to sunlight, only one compound has been shown to be affected by sunlight attentuation in the field. More field tests are needed in order to allow the interpretation of laboratory results.

Test a Wider Variety of Chemicals - A considerable amount of information is available about pesticides, detergents, and hydrocarbons but little information is available about other chemicals used in large commercial quantities.

Clarify Effect of Nutrient Recipes - A variety of nutrient recipes have been used either for convenience or for the ease of using a particular analytical method. The effect of varying the nutrients is little understood.

Determine Importance of Cometabolism as an Environmental Process - A number of researchers have considered to be biodegradable those chemicals that can be used as carbon and energy sources. However, this does not take into account cometablic processes which could be equally important and could alter the test approach considerably (add external carbon source).

Study Chemical Structural Relationships to Environmental Stability - Only a few studies were uncovered which attempted to correlate chemical structure to environmental persistence. More understanding along these lines would be extremely helpful. Few attempts have been made to correlate many physical properties of chemicals to their biodegradability.

Determine Kinetics of Various Metal Transformation Reactions in the Environment - Prior to doing this though, it might be necessary to understand the actual role of different biological systems in transformation. For example, at the present time it is normally assumed but not proven that microorganisms methylate metals which are then taken up by higher food chain organisms. Also more efforts should be made to find ways for predicting the ability of a metal to undergo methylation in the natural environment.

I. INTRODUCTION

A. General

Commercial development of chemicals has had a beneficial impact on the way man works and lives (ACS, 1973). However, in many cases, these commercial applications have resulted in an increased burden of chemical contaminants in the environment. The Council on Environmental Quality (1971) has suggested that "selected metals, their compounds, and certain synthetic organic chemicals are perhaps the best examples of toxic substances which can adversely affect man and his environment."

A major factor for assessing the potential hazard of these environmental contaminants is the consideration of the chemicals' environmental persistence and degradation products. For example, it is quite possible that "highly toxic, readily biodegradable substances may pose much less of an environmental problem than a relatively harmless persistent chemical which may well damage a critical wild species" (Goodman, 1973). This report reviews and evaluates techniques which have been used to determine the environmental persistence and degradation pathways of various chemicals.

The importance of knowing the alterations and ultimate fate of a chemical in the environment can vary depending upon the chemical. Understanding the environmental alterations of metals and organometallic compounds is required because the toxicity and mobility of the material may be greatly affected by various changes in chemical form. In addition, the kinetics of the alteration reactions in the environment may be extremely important since some of the degradation routes (e.g. methylation-demethylation) are reversible (Wood, 1974).

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With organic compounds, environmental reactions are usually irreversible and, furthermore, the organic compounds can potentially degrade to water and carbon dioxide. For organic compounds, the term "persistence" has much more relevance, and the kinetics of the reactions, although still quite important, have received somewhat less laboratory study than the qualitative assessments of whether a degradation process takes place or not. This is undoubtedly due to the fact that modelling the dynamic environment in the laboratory is most difficult in that the environment consists of numerous microenvironments which can vary considerably in temperature, pH, microbial population, etc. Thus, quantitative data developed under one set of laboratory conditions may be somewhat misleading for another set of environmental conditions, while the qualitative indications of environmental degradation may be relied upon in many different microenvironments.

Some definition of nomenclature is necessary. Such terms as environmental persistence, alteration, biodegradability and degradability are lacking in precision and are often dependent upon the perspective of the author. Soil scientists frequently refer to pesticides that are not retained in the soil for long periods of time as non-persistent, although, in many cases, the loss may be due to physical removal processes, such as vaporization and leaching (resulting in dilution but not destruction). Biodegradability is frequently considered by detergent scientists as synonymous with treatability or removability in a sewage treatment plant. The interpretation of these terms used in this report is briefly reviewed.

The terms persistent and degradable are applied in this report only to the total environment rather than any individual medium (air, soil, water) and, therefore, any transport processes of a chemical are irrelevant to a determination of persistence or degradability other than it may change the possible reactions that may take place (e.g., vaporization into the atmosphere almost eliminates the possiblility of biodegradation). Degradation means "to reduce the complexity of a chemical compound by splitting off one or more groups or larger component parts" (WPCF, 1967). Biodegradation means that the destruction process is accomplished by the action of living organisms (e.g. see Swisher, 1970). Environmental alteration means that the compound has undergone a chemical change that is not necessarily a reduction in the complexity of the molecule.

Biodegradation has been further divided into the following three categories (WPCF, 1967):

> "1. Primary Biodegradation - Biodegradation to the minimum extent necessary to change the identity of the compound.
>
> 2. Ultimate Biodegradation - Biodegradation to (a) water, (b) carbon dioxide, and (c) inorganic compounds (if elements other than C, H and O are present).
>
> 3. Acceptable Biodegradation - Biodegradation to the minimum extent necessary to remove some undesirable property of the compound such as foaminess or toxicity."

Primary biodegradation is basically the loss of the parent compound, although this is somewhat dependent upon the analytical methods used. Ultimate degradation is the complete mineralization of the organic chemical. The third category falls between the two extremes but results in perhaps the haziest definition. In another approach, Dagley (1972b) has suggested that a man-made compound is biodegradable if it is attacked by the enzymic apparatus acquired by microbes during the course of evolution. Painter (1973a) has noted that two general criteria have been used to assess the biodegradability:

(1) the rate and extent to which natural or enriched mixed cultures degrade the compound, (2) the proportion of tested species, strains or isolates which are able to use the compound as a sole source of carbon and energy. Prochazka and Payne (1965) feel that the ability to isolate microorganisms that use the test compound as a sole source of energy and carbon is the best criteria for biodegradability.

These definitions are obviously only of a qualitative nature. As mentioned earlier, this is due to the numerous chemical compounds to which the definitions are applicable and the lack of precision inherent in biological systems. This non-quantitative nature is in contrast to the half-life of an isotope, for example, where the rate is independent of environmental events (Dagley, 1972b). Thus, assessment of a chemical's persistence or degradation rate in the environment requires that the compound be evaluated in some type of test method or technique and the results are only meaningful when stated with the conditions of the technique. The components essential to such techniques are the test chemical, the analytical method, and the environmental component. A good test method should meet the following criteria: (1) be as simple as possible, (2) be as economical as possible, (3) be reproducible and (4) report results which can be correlated to field conditions (see SDA, 1965). These components of various techniques have been reviewed and evaluated in detail in the following sections.

Well over 9,000 synthetic organic chemicals are produced in commercial quantities (Council on Environmental Quality, 1971) and annually total approximately 140 billion pounds in the United States (U. S. Tariff Commission). Iliff (1972) has suggested that world-wide up to 40 billion pounds of manufactured organic chemicals enter the environment annually. Included are organic compounds used as pesticides (over 1 billion pounds, U.S. Tariff Commission), which are intentionally distributed in the environment, and detergents (Swisher, 1970; Anon., 1963), solvents (Levy, 1973; Laity et al., 1973), plastics (25 billion pounds in 1972), chemical intermediates (34 billion pounds in 1972), plasticizers (1.7 billion pounds in 1972) and other compounds, which may be inadvertently or accidently released during manufacture, transport, use, and/or disposal. Compounds, such as polychlorinated biphenyls (PCB's), phthalate esters, hexachlorobenzene, and other organic chemicals (e.g., Miller, 1973; Kleopfer and Fairless, 1972; Little A.D., Inc., 1970), are typical of the latter category. In addition, it has been estimated that more than 2 billion pounds of oil are lost at sea annually (Friede et al., 1972) and about 9.5 billion pounds of total plastic wastes accumulate each year (Potts et al., 1972).

These different categories of organic chemicals enter and reside in different parts of the environment. For example, detergents usually pass through a biological treatment plant before entering streams, rivers, lakes, etc. Pesticides are normally sprayed on crops or, if they are aquatic herbicides, they may directly enter aqueous systems. Those solvents which are fairly volatile vaporize into the atmosphere. From the initial entrance point, all of the above materials may be biologically or chemically altered or physically transported from one point to another. Understanding the mobility and physical transport mechanisms of a chemical is important in terms of determining the potential effect of the environmental contaminant (Oak Ridge National Laboratory under contract to EPA is reviewing techniques used to study the transport of chemicals in the environment). However, as long as the pollutant remains in existence, it can enter the food chain or affect man directly (Alexander, 1967). On the other hand, if the organic compound is completely mineralized to inorganic material (CO_2, H_2O), its potential for being a pollutant is removed. The possible biological and chemical degradation processes which may take place in the environment are reviewed in Section II (p. 33). In addition, biodegradation of chemicals under biological treatment conditions is considered since a compound's fate in these systems can be the determining factor in whether the compound becomes an environmental contaminant or degrades to innocuous material.

B. Methods and Approach

1. Literature Search

Papers, books and review articles relevant to this review were gathered by a variety of approaches. Extensive use was made of such abstracts as the Air Pollution Abstracts, Biological Abstracts, Bioresearch Index, Chemical Abstracts, Water Pollution Abstracts, and Pollution Abstracts. Since these abstracting services all have some amount of delay between when the article is published and when it appears in the abstract (approximately 3-6 months), a search of recent, highly relevant journals was also undertaken (October 1973 - March 1974). In addition, a computer search was performed of the reports published by the National Technical Information Service (NTIS) and the current investigator files of the Smithsonian Science Information Exchange (SSIE).

The abstracts, obtained in this way, were screened for relevant articles and the papers deemed pertinent were collected and examined. The bibliography of highly relevant articles was examined to provide a double check on the abstract search. In some instances, a citation search of extremely pertinent articles was conducted using Science Citation Index.

Individual researchers, who were either professional acquaintances of the authors of this report or were identified by the SSIE computer search, were personally contacted and requested to supply any recent articles they might have published or have in press. In addition, in a number of cases, personal visits were made to these researchers' laboratories in order to discuss experimental techniques used. Furthermore, a number of experts in the field have been asked to review drafts of the final report.

Unfortunately, no articles have reviewed the cost of the various techniques and, therefore, the prices that are quoted in this review are only estimates. Information which was used to calculate these estimates included (1) time requirements cited in papers, obtained from researchers, or estimated by the authors of this report, (2) complexity of the procedure and necessary technical training of the personnel, and (3) capital investments in equipment. The cost may fluctuate considerably depending upon the chemical being tested and the laboratory doing the test and, therefore, the projected costs have only an order of magnitude reliability.

2. Scope and Organization of the Report

This report reviews techniques that have been used to study the persistence and breakdown of chemicals in the water, soil, and air medium of the biosphere. With the exception of the model ecosystem technology, techniques used to study metabolism of chemicals in higher trophic levels (e.g., plants, fish, mammals) have been excluded. It was felt that these processes, although very important to considerations of bioaccumulation and toxicity, are less relevant to environmental degradation and persistence. A full discussion of this rationale is presented in Section II A. (p.33).

This report is organized in a variety of categorizations. In some instances, the type of chemical has determined the category (e.g., metals and polymers). In other cases, biodegradation is divided by different media (e.g., soil, water, sewage). In one instance, the type of degradation (chemical and photochemical) is the distinguishing factor. For the most part, these categories were chosen as a matter of convenience in reviewing the available information.

Most sections are divided into (1) a review of the techniques (2) analytical techniques (3) evaluation of the techniques, and (4) a cost analysis.

The quality and quantity of the information has varied considerably depending upon the chemical and the medium. By far, the most intensively studied group of chemicals in their appropriate medium have been the pesticides in soil and water, the detergents in sewage and water, and the hydrocarbons in the atmosphere. The approaches and techniques used with these materials have been reviewed for their applicability to other chemical groups of potential environmental contaminants.

Most of the techniques reviewed are applicable to a determination of degradability or persistence. Techniques for studying the routes of degradation have not been reviewed in a great amount of detail since they are very similar to procedures used to study metabolic pathways of natural substrates.

II. TYPES OF DEGRADATION IN THE ENVIRONMENT

Chemicals which are placed in the environment are subjected to chemical alterations and physical processes by sunlight, water, soil, inorganic and organic material, and biological agents and by the combined weathering action of rain, wind, temperature and humidity (Van Middelem, 1966). The physical processes, such as adsorption on colloidal substances, volatilization, bioaccumulation or leaching, have a tendency to distribute and dilute or concentrate but not destroy the contaminants. On the other hand, chemical and biological reactions in the environment result in alterations and frequently degradations of the material to innocuous substances. The alteration and degradation processes can be divided into three general categories: (1) biodegradation - effected by living organisms; (2) photochemical degradation - nonmetabolic degradation requiring light energy; and (3) degradation effected by chemical agents (chemical degradation) - nonmetabolic degradation which does not require sunlight. Alexander (1967) has suggested that of these three, biological degradation of organic compounds is the most desirable because it results generally in end-products that have been completely mineralized to inorganic compounds. In contrast, photochemical and other nonmetabolic processes usually result in only slight modifications in the parent compound. However, all of these processes are important from the standpoint of the environmental fate of the chemicals, and will be discussed in detail in the following sections.

A. Biological Degradation

In order to insure growth, maintainance and development, living systems must obtain carbon, other essential elements, and energy from their

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environment. For non-photosynthetic organisms, this process involves the absorption of various naturally occurring organic compounds, the subsequent metabolism of these compounds to yield energy and essential elements which can be incorporated into the structure of the organism, and the elimination of metabolic products. Although biological transformations of organic chemicals generally occur via relatively specific processes, there is sufficient latitude or non-specificity in many organisms to allow them to perform a significant role in the degradation and/or removal of various synthetic chemicals which are introduced into the environment. The factors governing this ability to degrade a natural or synthetic compound are: (a) the compound must be able to reach the organism or the enzyme site; (b) the compound must not be lethal; (c) the enzymes necessary to alter the chemical must be present or able to be induced; and (d) the environmental conditions must permit the operation of the enzyme(s) (Alexander, 1966). Thus, if absorbed by a given organism, a synthetic chemical may be (a) degraded to serve as a source of carbon and/or energy; (b) degraded by cometabolic processes (see Horvath, 1972a); (c) slightly modified and then stored or excreted; (d) stored or excreted without chemical alteration. Frequently there is a lag between the exposure of the chemical to the biological agent and the beginning of degradation or transformation. In the case of microorganisms, the lag is attributed to the need for "acclimation." This term will be frequently referred to in this report and, therefore, needs to be clarified. Acclimation is a broad term, usually referring to a variety of processes (such as enzyme induction, selection of a species of microorganism, etc.) which take place during the lag period.

Although biological degradation might conceivably be accomplished by any living organism, available information indicates that by far the most significant biological systems involved in ultimate biodegradation - degradation to CO_2, H_2O, and other inorganic compounds - are the bacteria and fungi. This conclusion is based on(1) what is known about the metabolic pathways of bacteria, fungi and higher organisms; (2) the projected metabolic activity of various organisms, and (3) the availability of synthetic organics to the bacteria and fungi. The following paragraphs are devoted to an elaboration of the above factors.

1. Metabolic Pathways

Various forms of life including wild life, fish, various marine species, domestic animals and even many have been shown to contain some residues of environmental chemicals (Smith and Isom, 1967; Davis and Hughes, 1965; Rawls, 1966; Durham, 1969; Edwards, 1973; Holden, 1970). Whether the eventual loss of chemicals from these organisms is due to metabolism or excretion is unclear. Loeffler and VanOverbeek (1971) have stated that "organisms with an effective excretory mechanism [which perhaps includes most animals] will, as a rule, not degrade the compound that does not fit into the normal sequence of metabolic events as completely as do those organisms in which this means of disposing of a compound is absent." This is based on the fact that a compound that is sufficiently water soluble (allows excretion) requires less energy for excretion than does the large number of reactions required to degrade a compound into small molecular fragments or even gaseous products (Loeffler and VanOverbeek, 1971). On the other hand, water insoluble compounds, e.g., chlorinated hydrocarbons, insecticides, tend to accumulate, and storage of residues often occurs in the adipose tissue of the organism (Edward, 1973; Kenaga, 1972).

Higher plants usually do not have an efficient excretion apparatus. The metabolic tendency in such cases is to convert the chemical into some neutral, water-soluble form in which it can be stored in cell vacuoles (Crosby, 1973). Examples of these transformations prior to storage include (1) conversion of naphthaleneacetic acid to its glucose ester, followed by the formation of the more stable amino acid conjugate (Veen, 1966), (2) conversion of the insecticide carbaryl into persistent glycosylated metabolites (Kuhr and Casida, 1967); and, (3) binding of certain pesticides to plant proteins (Brian, 1960) or incorporation into structural lignins, pectic substances, etc. (Chin et al., 1964; Meagher, 1966).

In contrast to the metabolic processes of high organisms, the catabolic versatility of bacteria and fungi suggests that these organisms are likely to play a major role in the ultimate degradation of synthetic chemicals which enter the environment. Alexander (1973a) has stated that all evidence points to the fact that microorganisms are responsible for converting to inorganic products many complex natural and synthetic organic molecules which cannot be significantly altered by higher life forms. Enzymatically, the degradative activity of bacteria and fungi may be based on their ability to catalyze the initial steps in degradation resulting in metabolites that can enter existing metabolic pathways (Dagley, 1972a). Furthermore, microbial communities in the environment seem to vary more in the number of different microbial groups present. This high species diversity in microbial communities results in a multitude of possible biochemical pathways which may provide a major advantage to microorganisms over other organisms in catalyzing degradation of the many structurally different environmental chemicals.

Invertebrate forms including the protozoans and lower metazoans have not been as intensively studied as the vertebrates, higher plants, bacteria, and fungi. These invertebrates have not as yet been directly implicated in the ultimated degradation of synthetic chemicals although they are associated with the dispersion of such compounds (Wright, 1971) and thus make the chemicals more accessible to the bacteria and fungi (Alexander, 1966).

A great number of studies can be cited in which a compound has been found to be degraded by the action of microorganisms but not by higher organisms. Funderburk (1969) has reported no metabolism of paraquat in higher plants. Numerous microorganisms have, however, been found to use paraquat as a sole source of carbon or nitrogen (Baldwin *et al.*, 1966; Anderson and Drew, 1972). DDT, a pesticide well known to be persistent in organisms of higher trophic levels (Edwards, 1973), has recently been shown to undergo extensive degradation by the combined action of two bacteria, as shown by *in vitro* studies of Pfaender and Alexander (1972). Another good example is the herbicide endothal. Sikka *et al.* (1974) have shown that blue gills removed less than 1% of the total amount of herbicide; the removed amount was present in the fish in unchanged form. Shake culture and aquarium studies (Sikka and Saxena, 1973; Sikka and Rice, 1973) have, however, shown that endothal is rapidly degraded by microorganisms. When mixed function oxygenases from liver microsomes (from animals) attack naphthalene, an epoxide is formed which may isomerize to give naphthol, or undergo enzymic hydrolysis to the *trans*-dihydrodiol; the product formed is usually excreted as conjugates (Gibson, 1972; Dagley, 1972b). By contrast, microorganisms oxidize naphthalene and break down the rings to provide a source of carbon and energy for growth (Gibson, 1972; Dagley, 1972b).

Degradation of several natural materials by microorganisms can further substantiate the unique role of microorganisms in governing environmental persistence of chemicals. Pristane, an isoprenoid alkane which is formed by crustaceans of the genus Calanus, has been found to accumulate unchanged in the locations where it is not susceptible to microbial attack (McKenna and Kallio, 1971; Dagley, 1972b). Under favorable conditions, however, pristane is readily metabolized by a variety of microorganisms (McKenna and Kallio, 1971; Dagley, 1972b). Another natural product, atropine, which is probably even more toxic than many pesticides, has been found to serve as a source of carbon for certain microorganisms (Niemer *et al*., 1959; Niemer and Bucherer, 1961). In other living forms, these compounds evoke mechanisms of detoxification and elimination (Dagley, 1972b).

This relative metabolic advantage of microorganisms over high organisms might be attributable to the difference in their evolutionary processes. Microorganisms, for the most part, reproduce more rapidly and have a high rate of mutation, thus allowing them to develop enzyme systems over the years that will metabolize a greater variety of organic structures. However, the diversity in microbial communities which provides a multitude of possible biochemical pathways is probably even more important.

2. Metabolic Activity

The amount of material consumed by any given group of organisms will depend largely on the product of their biomass and metabolic rate/unit mass. In general, the rate of metabolism increases with increasing surface/volume ratio (Thimann, 1966).

Organism	Surface/volume ratio
200-lb. man	0.3
Hen's egg	1.5
Amoeba	400
Bacteria (0.5μ or less)	120,000

The bacteria and fungi may thus be expected to be immensely more metabolically active per unit weight than higher life forms. For example, a lactose-fermenting bacterium will break down from 1000-10,000 times its own weight in lactose in one hour, whereas a man would require 250,000 hours to break down 1000 times his own weight in sugar (Thimann, 1966).

An analysis of the metabolic activity of different organisms in soil by Macfadyen (1957) has demonstrated the importance of bacteria in a limited ecosystem (see Figure 1). The data indicate that bacteria alone account for the major portions of the total metabolism in the soil.

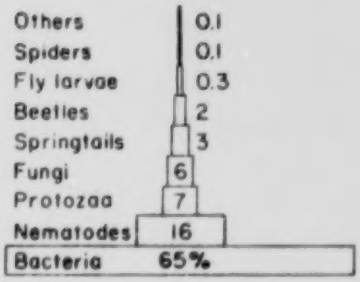

Figure 1: Percent Total Metabolism by Different Taxa of a Hypothetical Soil Community in a Meadow (Macfadyen, 1957) Reprinted from *Animal Ecology, Aims and Methods*, Copyright Pitman Publishing.

This can be attributed to a much larger magnitude of biomass and higher metabolic rates of soil microorganisms compared to soil animals (Table 1) (MacFadyen, 1963). Dagley (1972b) has also estimated that microbial biomass (including algae) is much greater than that of the combined animal biomass.

This approach of analyzing ecosystems on the basis of metabolic activity is particularly relevant to an assessment of the relative importance of various taxa in that it illustrates the relative rates of energy consumption, which is related to organic material consumption.

Table 1: Figures From a Hypothetical Grasslands Soil Community Showing Order of Magnitude of Numbers, Biomass, and Metabolism for the Main Biological Groups (Macfadyen, 1963)

Group of Organisms	Weight of Organism (mg)	Approximate estimates grassland per sq m Numbers	Mass (g)	Metabolism Calories per day per g at 16°C
Bacteria	10^{-9}	10^{15}	1000	575
Fungi			400	161
Protozoa	0.05	5×10^{8}	38	14
Nematoda	0.001	10^{7}	12	144
Lumbricidae	5000	10^{3}	120	7
Enchytracidae	0.14	10^{5}	12	100
Mollusca	1500	50	10	29
Myriapoda	25	500	12.5	36
Isopoda	22.7	500	5	36
Opiliones	18	40	0.4	53
Acari-				
Parasitids	0.2	5×10^{3}	1.0	280
Oribatei	0.25	2×10^{5}	2.0	72
Araneae	10	600	6.0	27
Coleoptera	250	100	1.0	39
Diptera	610	200	1.0	29
Collembola	0.46	5×10^{4}	5.0	144

3. Availability of Synthetic Organics to Various Taxa

Even given the high order of metabolic activity and versatility shown by the bacteria and fungi, their role in biological degradation might be somewhat limited if significant amounts of synthetic organic chemicals were stored by higher organisms, thus making the chemicals unavailable for microbial degradation. However, such a situation does not seem likely in the environment. For example, Edwards (1973) has calculated the amount of organochlorine insecticides which is locked up in the soil biota. The calculations are based on the average figure of 1.0 ppm of organochlorine residue in the living organisms. Considering that there is an average of 25 tons of living organisms per ha (Stockli, 1950), the amount stored in the biota will be equivalent to 0.025 Kg/ha (0.022 lb./acre) (Edwards, 1973), which is in the range of 1-2% of total (of many herbicides) normally applied per acre (application rate: 1-2 lbs. actual/acre, Thomson, 1967). DDT may be typical of this group of insecticides. Woodwell and coworkers (1971) have calculated that the total biota in oceans, fresh water and soil probably contains 3.3% or less of the amount of DDT produced in one year (during the mid-1960's). Compatable with this projection, The Study of Critical Environmental Problems (SCEP, 1970) estimates that of the 500,000 tons of DDT existing in the worlds oceans, only 0.12% is stored by fish. Although the data presently available is somewhat fragmentary, it does suggest that synthetic organic chemicals which are not readily degradable by higher life forms are also not stored in significant amounts in higher life forms and are thus available for microbial degradation.

Thus, the bacteria and fungi seem to serve and will probably continue to serve as the major factors in the ultimate biodegradation of synthetic organic chemicals in the environment. Consequently, this review has focused on techniques which utilize for the most part microorganisms as the biological agents of degradation.

B. Photochemical Degradation

The photolysis of a chemical in the environment appears to be an important process which may have a considerable effect on a chemical's persistence and degradation. This is especially true of atmospheric contaminants (Altshuller and Bufalini, 1971; Leighton, 1961) and many organic pesticides (Crosby and Li, 1969; Crosby, 1969a,b; Plimmer, 1970).

In order for a chemical to react photochemically, it must be able to derive energy from the incident light available to it. In the environment, sunlight is the incident light source. The ozone in the atmosphere affectively absorbs all sunlight of wavelengths less than 290 nm (Koller, 1965) and for practical purposes, the energy available for photochemical transformations does not exceed 95 kcal/mole (300 nm) (Crosby and Li, 1969). As the wavelength gets longer, the energy available for photochemical change is reduced. Crosby and Li (1969) have suggested that wavelengths longer than 450 nm (65 kcal/mole) would not be expected to bring about chemical changes under most circumstances (see Figure 2). Thus, in terms of environmental photolysis, processes that take place upon irradiation with light between 300-450 nm are the most significant.

The theory of how a chemical absorbs and chemically utilizes the energy from light has been adequately reviewed elsewhere (Calvert and Pitts, 1966; Owen, 1971). However, some very basic fundamentals will be briefly reviewed in order to provide perspective to the experimental techniques that will be discussed.

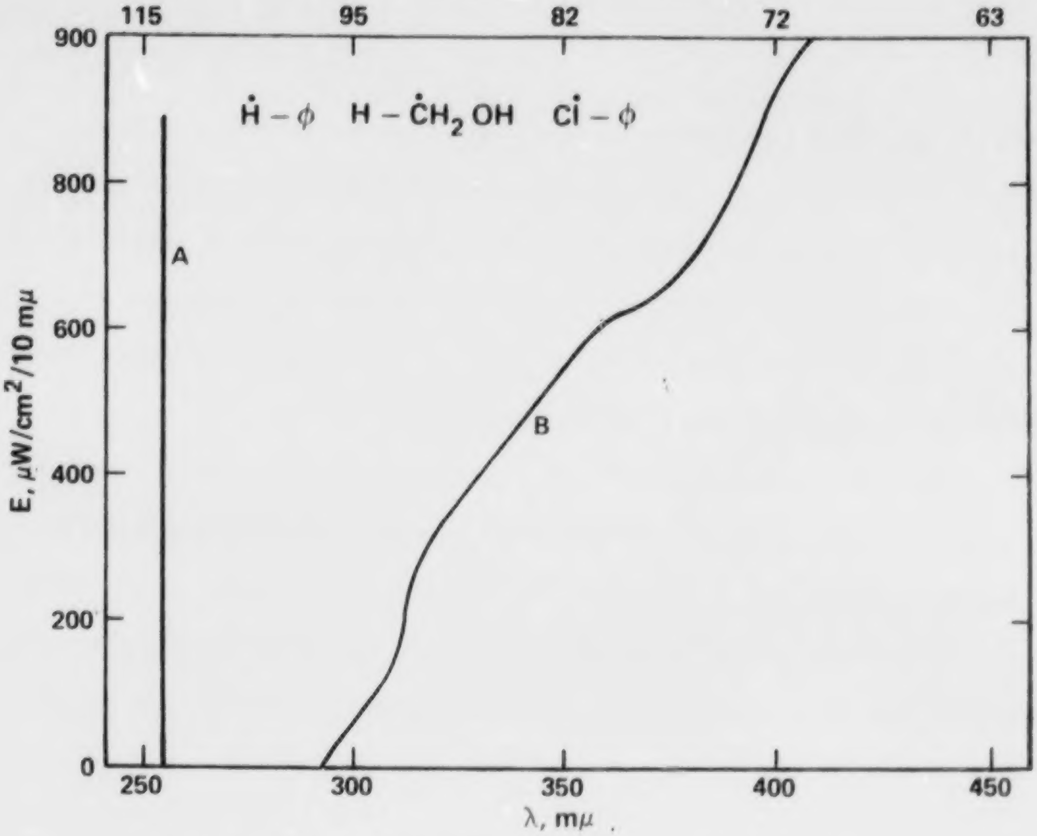

Figure 2: Spectral Energy of Sunlight (B) Relative to Chemical Bond Energies and Low-Pressure Mercury Arc Lamp (A) (modified from Crosby, 1969b and Crosby, 1972a)

A molecule may become excited by direct absorption of light or by accepting energy from an excited donor molecule (sensitizer). The ultraviolet absorption spectra provides an indication of the ability of the molecule to absorb energy at various wavelengths (extinction coefficient), and that is dependent upon whether the transition is "allowed" (determined by selection rules, see Jaffe and Orchin, 1962). This absorption is very dependent upon the matrix of the molecule (vapor phase, absorbed on a solid, dissolved in various liquid). In fact, it is quite possible that a compound that does not absorb at >290 nm in a solvent such as hexane, may absorb light in the environment. For example, Plimmer (1972b) has noted a 60 nm red shift for trifluralin (a nitroaromatic herbicide) when it was adsorbed on silica gel. Sensitization occurs when an acceptor molecule has an excited state of correct energy relative to a donor molecule and it requires some interaction between the donor and acceptor.

Once the molecule is excited, it can release its energy in several non-chemical ways (Figure 3) including fluorescence and phosphorescence (irradiative decay) and radiationless decay (energy transferred to the matrix). On the other hand, the molecule may use the energy to chemically alter the molecule. The efficiency of each of these processes (usually stated as quantum yield) is again somewhat dependent upon the matrix.

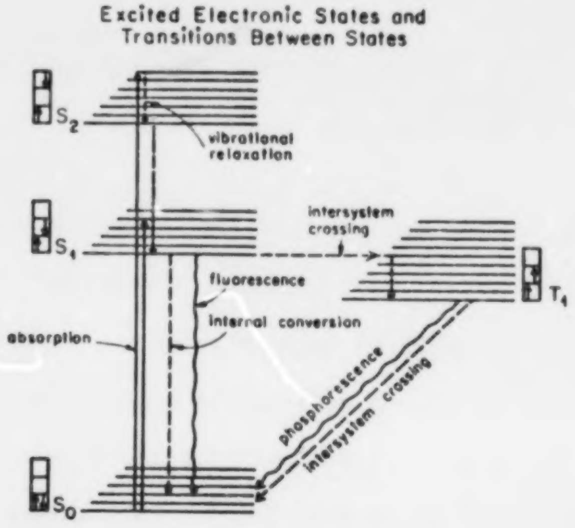
Excited Electronic States and Transitions Between States
S_2
vibrational relaxation
intersystem crossing
S_1
T_1
fluorescence
internal conversion
absorption
phosphorescence
intersystem crossing
S_0

Figure 3. Modes of Formation and Deactivation of Molecules in the Excited State (Owen, 1971). Reprinted from Organic Chemicals in Aquatic Environments, by courtesy of Marcel Dekker, Inc.

Photochemical alteration can also occur by the interaction of the compound of interest with a photochemically excited molecule (e.g., singlet oxygen, Plimmer, 1972a, Crosby, 1972b; or diethylaniline with DDT, Miller and Narang, 1970). Again the neighboring molecules are extremely important to the process.

Because of these effects which are induced by the matrix, laboratory simulation of environmental photolysis is very difficult. The conditions that are used are sometimes very remote from natural conditions and the results obtained should be extrapolated with a great deal of caution.

C. Degradation by Chemical Agents

Other nonmetabolic decompostion processes besides photolysis may also be important in the environment (Rosen, 1972 a; Crosby, 1970; Crosby, 1969a). Ubiquitous substances, such as air, water, soil, etc. may react with or catalyze environmental degradation or alteration reactions. Unfortunately, with the exception of hydrolysis processes, relatively few non-photolytic chemical (as opposed to biological) reactions are known (Rosen, 1972a). Crosby (1970) has suggested that this low number of reported chemical reactions, at least in soil, is due to a lack of distinction between biological and nonbiological reactions in experimental studies. Such differentiation is extremely difficult because of the difficulty of sterilizing soil without changing the soil structure and chemical composition. In contrast, this distinction between the two processes (biological and chemical) is extremely simple in reactions that take place in the atmosphere since biological processes are minimal. Some of the major non-photolytic chemical processes in the environment will be briefly reviewed.

The free-radical character and solubility in water make oxygen a most effective environmental reactant (Crosby, 1970). Many compounds upon exposure to air will be readily oxidized. In many cases the presence of light accelerates the oxidation process but a considerable number of reactions can take place in the dark (Crosby, 1969a).

Pesticides such as phosphites, sulfoxides and dithiocarbamates are readily oxidized to phosphates, sulfones and thiuram disulfides, respectively. Oxidation reactions are particularly facilitated in the atmosphere (Haagen-Smit and Wayne, 1968) where the medium consists of 20% oxygen. Other oxidation reactions initiated by oxidants or free radicals formed in photochemical smog may also be important to the environmental fate of a chemical substance (Crosby, 1969a).

Water, besides being an important medium for chemical reactions, is reactive itself through its ability to form either hydroxyl or hydrogen ions. Consequently, pH becomes important in nonmetabolic decompostion of chemicals in an aqueous medium. Normally drinking water pH is between 5 and 8, although values as high as 10.5 have been noted (Crosby, 1969a). In soil, the water is seldom neutral and can vary from a pH of 3 to 10.5 (Crosby, 1970). Although solvolysis and elimination reactions have been noted (Rosen, 1972a). Crosby, 1969a), the most widely recognized and studied reaction with water is hydrolysis. The reaction is usually pH dependent and can be catalyzed by various agents (e.g., hydrolysis of parathion is catalyzed by common amino acids, hydroxylamine derivatives, metal ions, and metal ion chelates). With organophosphorus esters, the hydrolysis reaction mechanism is different under acidic or neutral conditions (alkyl-oxygen bond is attacked) compared to under alkaline conditions (phosphorus-oxygen bond attacked).

Many chemical substances that enter the environment become associated with soil and dust particles. Some mineral constituents of soil, such as clays, can act as catalysts. For example, they can cause endrin to isomerize from an epoxide to a ketone. The organic constituents of soil can serve as oxidizing and reducing agents since they contain a relatively high concentration of stable organic free radicals.

All the above processes can be accelerated by an increase in temperature and, therefore, temperature is an important degradation parameter.

In general, non-photolytic reactions effected by chemical agents have received considerably less attention than other environmental processes. However, it should be kept in mind that in many systems used to study biological or photochemical degradation, degradation by chemical agents cannot be excluded.

III. BIODEGRADATION OF CHEMICALS IN AQUATIC OR SEWAGE TREATMENT CONDITIONS

Organic chemicals entering the aquatic environment can be degraded by chemical or biological agents. Degradation techniques which are used to study processes effected by chemical agents are reviewed in Section V. p. 283. In this section, the more frequently used biodegradation techniques are reviewed. The section has been divided into two major subdivisions; (1) static tests which use low bacterial concentrations and simulate natural water systems, and (2) dynamic systems which maintain high concentrations of bacteria and thus more closely simulate conditions of a biological waste water treatment plant.

A. Techniques for Determining Biodegradation of Chemical Compounds in the Aquatic Environment

Chemical compounds can enter water in a variety of ways. These include runoff from land, discharge of industrial waste, home use and garbage disposals (sewers), dumped through carelessness and accidents, and direct application to water to control pests. Oceans are contaminated by oil at an estimated rate of 5 million tons per year (Blumer and Sass, 1969; Blumer et al., 1973). Chemicals can also enter the marine environment due to run-off from treated lands into the rivers and finally into estuaries and oceans. Even when present at low concentrations, these compounds may be hazardous since a large number of them can bioaccumulate in the food chain.

The origin of microorganisms in the aquatic environment is not clearly understood. It has been suggested that the variety of microorganisms present in natural waters is of allochthonous origin; e.g., they are introduced from the washings from soil or with sewage (Wuhrmann, 1964). Specialized types of microorganisms may be found in a stream due to the unusual chemical conditions with regard to its inorganic and organic composition. The environmental conditions

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in the aquatic system differ in many other respects from those of soil and sewage. The differences, which include concentration and types of organic chemicals, microbial community, supplemental nutrients, light conditions, etc., can significantly affect both the extent and rates of biodegradation. Natural water represents an extremely diluted medium where continuous replacement of the metabolic substrates and removal of end products takes place due to the flow of water.

A number of methods have been developed to ascertain the persistence or decomposition of chemical compounds in the aquatic environment. The compounds normally investigated for their persistence in the aquatic environment include surfactants, pesticides, oil pollutants and certain chemicals resulting from industrial synthesis. Biodegradation is generally measured by exposing the test compound to microorganisms and analyzing periodically for the disappearance of the intact test compound, the formation of degradation products, or the susceptibility to oxygen uptake. Thus, three components are essential to an organic chemical biodegradation test system: the chemical compound, the biological agent, and the analytical method.

The choice and the source of biological agent and environmental conditions used in the test method are among the important factors that influence biodegradation. The classification of test methods described in this section has been aided by consideration of these factors. As a result of variations in these components of the test system, investigators have devised tests varying from such simple test methods as the river die-away test to more complex systems such as Metcalf's aquatic ecosystems. In certain instances, if a particular analytical method (e.g., biochemical oxygen demand) has been so widely used that it is more or less regarded as a biodegradability test method, it has been considered as a separate class.

1. Biochemical Oxygen Demand

Many organic compounds are degraded by natural microbial communities in the presence of oxygen--which usually results ultimately in the conversion of the substrate acted upon to CO_2, water, and other inorganic products. Consequently, measurement of oxygen uptake has proved to be a useful method to estimate the extent of biodegradation. However, interpretation of the oxygen measurement result is somewhat difficult since microorganisms oxidize organic matter to carbon dioxide and water while simultaneously also synthesizing new cell material, metabolites, etc., which have oxidation states different than that represented by carbon dioxide and water. In most instances, therefore, the measured biochemical oxygen demand is considerably less than the amount of oxygen needed for complete oxidation (calculated theoretically).

Several methods which have been used for determination of biochemical oxygen demand can be grouped into the following categories:

a. Dilution Method

The BOD test consists of measuring the depletion of dissolved oxygen during or after a period of incubation. The dissolved oxygen level is normally measured either by chemical methods, or by the use of an oxygen-sensitive electrode. The oxygen available for degradation is limited to the amount which is dissolved in the water at the start in the test (8-10 ppm, Swisher 1970). This limits the initial concentration of the test compound as well as the size of inoculum in the test system (to obtain the desired range of oxygen depletion in a given period). The temperature is usually maintained at 20°C, which is similar to the temperature of surface waters during summer.

(i) Standard 5-day BOD procedure

The procedure is described in detail in the standard manual of the American Public Health Association (APHA, 1971, p 489). The test uses the incubation bottles of 300 ml capacity having a special shape designed to hold a water seal so that oxygen transfer from the atmosphere into the bottle will be minimal. Dilutions are prepared for each test chemical so that oxygen depletions fall in the range of 2 to 7 mg/ℓ (convenient range for measurement).

In the standard BOD tests, the dilution water which contains necessary trace nutrients for proper growth of microorganisms is prepared (APHA, 1971, p 489), and then the water is seeded at a concentration of 1-2 ml/ℓ of dilution water with settled domestic sewage which has been stored at 20°C for 24-36 hours. In certain instances specialized seed material containing organisms adapted to the use of the organic compounds may also be used. Such adapted seed is often obtained from the effluent of a biological treatment process receiving the waste in question. Adapted seed may also be developed in the laboratory by continuously aerating a large sample of water and feeding it small daily increments of the test chemical together with soil or domestic sewage.

The effect of various seed types on BOD results is shown in Table 2.

Table 2. Effect of Seed Type and Quality on BOD Results (APHA, 1971)

Type of Seed	5-day Seed Correction* mg/ℓ	Mean 5-day BOD** mg/ℓ	Standard Deviation mg/ℓ
Settled fresh sewage	>0.6	218	±11
Settled stale sewage	>0.6	207	± 8
River water (4 sources)	0.05-0.22	224-242	±7-13
Activated-sludge effluent	0.07-0.68	221	±13
Trickling filter effluent	0.2-0.4	225	± 8

* BOD in the absence of added carbon source

** For Standard Solution (150 mg each of glucose and glutamic acid)

The bottles are incubated for 5 days in the dark at 20°C and then the dissolved oxygen in the samples is determined using an iodometric method or a membrane electrode. A summary of variations employed in the test method as used by various investigators is given in Table 3.

For this test the American Public Health Association has recommended that the quality of the dilution water, the effectiveness of the seed and the technique of the analyst should be checked periodically using pure organic compounds on which the BOD is known or determinable. For this purpose a solution containing 150 mg/ℓ each of reagent grade glucose and glutamic acid can be used. The 5 day BOD value for the Standard Solution may vary slightly according to the type of seed used (Table 2). If, however, the results vary appreciably from those given in Table 2 after considering the seed source, the technique is questionable.

Table 3. Summary of Standard 5-day BOD Procedure Used by Various Investigators

Reference	Compound tested	Concn.	Type of Seed
Bogan and Sawyer (1955) Sawyer et al. (1956)	Synthetic detergents	-	Natural sewage, Acclimated activated Sludge
Ryckman and Sawyer (1957)	Surfactants	-	Acclimated activated Sewage
Conway and Waggy (1966)	Surfactant	3-10mg/ℓ	Acclimated laboratory activated sludge
Pfeil and Lee (1968)	Nitrilotriacetic acid (NTA)	0-320mg/ℓ	Raw sewage aged for 24 hours, Activated sludge, Acclimated activated sludge
Thompson and Duthie (1968)	NTA	2 and 5 mg/ℓ	Seed acclimatized to NTA in BOD water for 14 days
Dias and Alexander (1971)	Aliphatic acids and alcohols[1]	2mg carbon/ℓ	Domestic sewage aged for 24-48 hours
Sturm (1973)	Nonionic Surfactants	2 and 5 mg/ℓ	Sewage microorganisms acclimatized to test chemical and yeast extract (2mg of carbon/ℓ)

[1] temperature 25°C; each compound was also tested in combination with glucose

(ii) 10-Day BOD Procedure

Mills and Stack (1954) have described a 10-day BOD test and used it for studying biological oxidation of synthetic organic chemicals. The reason for using a longer incubation period was due to the occurrence of a lag period of one to two days before oxygen depletion was observed in control bottles (without the chemical). These investigators inoculated the dilution bottles with dispersed seed (Heukelekian, 1949) developed from settled sewage which had been acclimated. The desirable characteristics of the seed included insignificant adsorption of the chemical and ready availability of the seed for batch experiments. The method has also been referred to as the "dispersed seed aeration system" (Mills and Stack, 1954). Another modification made in this test was that air containing 1% CO_2 was used for aeration whereas in the standard BOD test, air is used. This was done to control the pH of the mixture being aerated by preventing the loss of CO_2.

(iii) Long-term BOD Technique

This technique was developed by Elmore (1955) for determining BOD when (a) the compound requires long acclimation periods (b) the compound requires long periods for complete degradation, or (c) higher concentrations of the test chemicals are used. In this test a large bottle (several liters capacity) is filled with the test solutions and aerated. From this a series of several standard BOD bottles are filled and incubated.

The large bottle is also incubated. On subsequent days a single bottle is removed and analyzed for dissolved oxygen. When all the small bottles have been analyzed or when the dissolved oxygen level is near exhaustion, the large bottle is reaerated and from this another series of small bottles is prepared for analysis on subsequent days. This procedure is repeated as many times as necessary until the degradation of the chemical is complete.

(iv) Two-Bottle - Single Dilution Reaeration Method

This method was originally described by Orford et al. (1953) and later used by Ryckman (1956), Ryckman and Sawyer (1957), Young et al., (1968) and Buzzell et al. (1968). It permits withdrawing many samples for analysis and has provision for replenishment of oxygen as it is depleted.

The biodegradation reaction unit consists of two 9-liter pyrex bottles with connecting siphon as shown in Figure 4. The lower bottle is kept full and when the samples are withdrawn from this bottle for dissolved oxygen analysis, the second bottle replenishes the liquid through the siphon.

Similar to other BOD tests, the dilution water is inoculated with raw sewage to provide seed for biodegradation. If necessary, the contents of both bottles may be reaerated by directing air through the sampling tube.

Buzzell et al. (1968) expanded the Two Bottle - Single Dilution Reaeration Method to make it more nearly quantitative and more general in nature. These researchers measured the following parameters in the sample removed from the biodegradation reaction unit:

Utilization of oxygen to determine BOD, chemical oxygen demand, and total organic carbon to monitor removal and transformation of carbon.

Changes in bacterial population numbers to assess the deleterious effect of the chemical, if any, or utilization of carbon from the organic compound.

Onset of nitrification to estimate noncarbonaceous oxygen utilization.

The total information obtained above was considered in assessing the behavior of organic chemicals.

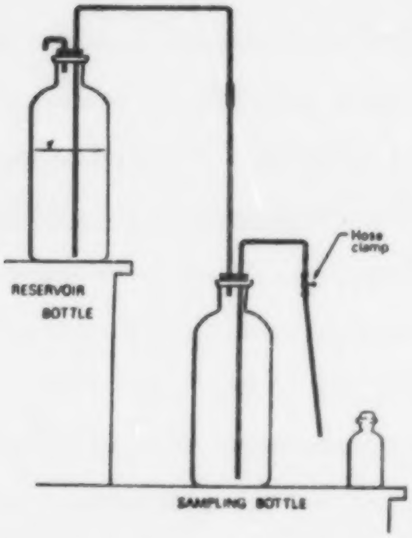

Figure 4. Schematic Representation of Individual Biodegradation Reaction Units (Young et al. 1968) Reprinted with permission from Journal Water Pollution Control Federation, Vol. 40(8 Part 2), 354-368, Wash., D.C.

b. Respirometry

Similar to the dilution method, respirometric technique determines biodegradability by measuring oxygen consumption linked to the oxidation of the test chemical. The methods described in this section, however, differ from standard dilution methods in that no serial dilution of the test sample is necessary. The oxygen consumption measurement in the respirometric method is generally made for a period much shorter than 5 days. The seed used in the respirometric test has generally been acclimated to the test chemical whereas, in the BOD dilution method, acclimation is assumed to occur during the test period. Since the concentration of microorganisms used in respirometric methods is generally higher, treatment plant conditions may be simulated more closely. Furthermore, unlike the standard dilution method, which measures oxygen consumed at 5 day intervals, the respirometric technique allows continuous measurement of oxygen uptake. The measurement can, therefore, be stopped as soon as the oxygen consumption rate has leveled off.

The respirometric technique used for studying biodegradation can be divided into two categories depending on whether or not sewage (or external carbon source) is oxidized along with the organic compound of interest.

> Systems containing organic compound, external carbon source and microorganisms. The concentration of the external carbon source is normally high while the concentration of the test organic compound is kept low. Three respirometric runs are made: one with microorganisms alone, one with microorganisms and external carbon source, and one with microorganisms, external carbon source and the test organic compound. The advantage of this technique is that sewage could supply organic matter to those

microoganisms that can oxidize the organic compound but not utilize it for carbon and energy. A problem of interpretation of results may arise in this method because the organic matter present may effect the decomposition of the test compound. Therefore, the method is not frequently used.

For studies of the degradation of surfactants, the common procedure is to use concentrations of suspended solids and sewage which approximate the concentrations found in activated sludge systems. The surfactant concentration is kept as low as would be found in sewage entering a treatment plant (usually about 100 mg/ℓ or somewhat higher) (Bogan and Sawyer, 1954; Barden and Isaac, 1957; Hunter and Heukelekian, 1964).

System containing organic compound and microorganism. In this technique only two respirometric runs are required: one containing microorganisms only and one containing microorganisms and the test organic compound. External carbon source or sewage is not added and therefore the system is less complicated. For this reason, the procedure is often preferred for the determination of biodegradability (Vath, 1964; Bogan and Sawyer, 1955; Sawyer et al., 1956; McKinney and Symons, 1959; Blackenship and Piccolini, 1963). If sludge is used as the source of microorganisms, unassimilated organic matter is removed prior to its use as seed in the respirometer. This can be accomplished either by not feeding the sewage several hours preceeding its use, thus forcing the microorganisms to burn unassimulated carbon (Bogan and Sawyer, 1955), or by washing the sludge with saline solution prior to its use in respirometry (Blackenship and Piccolini, 1963).

The type of inoculum used in respirometric studies has varied considerably. Researchers have used both mixed and pure cultures of microorganisms. The use of the mixed population of microorganisms has been more frequent since they more closely represent natural environmental conditions. Sources of mixed population have included sewage, activated sludge, trickling filter slime, river water, and river or lake mud (Hunter and Heukelekian, 1964). Pure cultures for respirometric studies have generally been isolated by enrichment culture technique (see section III A.3.b.iii., p. 105) or obtained from stock cultures of microorganisms (Heyman and Molof, 1967; Ellis et al., 1957; Walker and Cooney, 1973a and b).

The duration of the respirometric test is relatively short and acclimation of the organisms to the chemical may not occur during this period. Consequently, microorganisms acclimated beforehand are often used. Ryckman and Sawyer (1957) found that degradation of alkylbenzene sulfonates was delayed for several days when organisms native to sewage were used. However, when acclimated activated sludge was used, degradation was rapid. Activated sludge from a unit acclimated to the surfactant has been used as the inoculum for studying degradation of surfactants (Bogan and Sawyer, 1954, 1955; Sawyer et al., 1956; Barbaro and Hunter, 1965; Nelson et al., 1961; Brink and Meyers, 1966). Okey and Bogan (1965) used cultures which were derived from soil and sewage inoculum and grown on unsubstituted homologs of the chlorinated test substrates. In other instances, they used the activated sludge grown on a homologous substance. In order that the seed used in one laboratory can be easily duplicated elsewhere, Buzzell et al. (1969) used activated sludge, acclimated to the synthetic sewage which had been preserved by lyophilization (freeze drying). The microorganisms were reactivated by mixing dried sludge with synthetic sewage prior to use in the Warburg apparatus.

A number of techniques have been devised for respirometric studies. Some measure gas exchange by manometric means, e.g., Warburg respirometer. Others measure oxygen by means of polarography, e.g., oxygen electrode. Researchers have modified these systems according to their requirements. Various respirometric techniques and their modifications are discussed below.

(i) Warburg Method

In this test, oxygen is supplied to the system in the form of the gas phase which is continuously transported into the liquid phase by agitation. The Warburg respirometer determines oxygen uptake by measuring pressure changes while the gas and liquid volume are held constant. The higher level of microbial activity which is normally obtained in a respirometer flask simulates more nearly a biological treatment unit rather than a lake or a river.

A Warburg respirometer unit is shown in Figure 5. It consists of a U-shaped capillary tube of uniform cross section, both arms of which are graduated in millimeters. Attached to one arm is a sample vessel. In a typical respiration experiment, the flask contains an oxygen containing gas phase, a liquid phase containing an organism and its substrate, and a center well containing potassium hydroxide solution to absorb produced CO_2. The flask is immersed in a water bath at constant temperature and shaken or swirled to promote a rapid gas exchange between the liquid and the gas phase. From the magnitude of the change, which is indicated by the difference in the height of the fluid in one arm of the manometer, the quantity of oxygen consumed can be calculated.

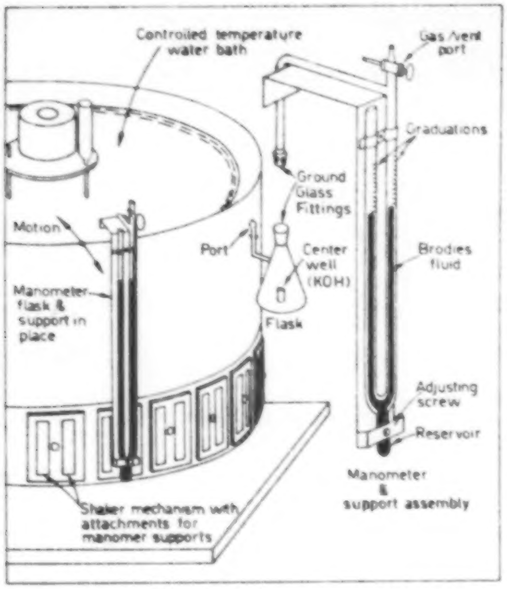
Controlled temperature water bath
Gas/vent port
Graduations
Ground Glass Fittings
Motion
Port
Center well (KOH)
Brodies fluid
Manometer flask & support in place
Flask
Adjusting screw
Reservoir
Manometer & support assembly
Shaker mechanism with attachments for manomer supports

Figure 5. Warburg Manometer
(Eckenfelder *et al.*, 1972)
Courtesy of Springer-Verlag

Changes in the barometric pressure are compensated for by the use of a blank flask containing water, which is known as the thermobarometer. The extent of endogenous oxygen uptake is estimated from a control run which is identical to the test run except that the test compound is not added.

Summary of the Warburg conditions used by various investigators is given in Table 4:

Table 4. Warburg Respirometry Conditions

Reference	Test Chemical	Concentration used	Period of Incubation
Bogan and Sawyer (1955)	Synthetic detergents	50-100 ppm	6 hours
Sawyer *et al*. (1956)	Synthetic detergents	50-100 ppm	6 hours
Steinle *et al*. (1964)	Surfactants containing ethylene oxide monomers	-	48 hours for poorly degradable compounds 8-10 hours for easily degraded compounds
Okey and Bogan (1965)	Hydrocarbons	50 mg/ℓ 1 mg/ℓ (substrate containing chlorine)	10-72 hours
Brink and Meyers (1966)	Anionic surfactants	-	12-24 hours
Heyman & Molof (1967)	Surfactants	0.01-0.005 M Primary alkylbenzene sulfonate 0.005M secondary linear compounds	2-26 hours

(ii) Modification of Warburg Apparatus

Caldwell and Langelier (1948) modified the Warburg apparatus to accommodate large samples (10-75 ml instead of 3 ml). They increased the volume of the reaction flask to 125 ml. Gellman and Heukelekian (1951) used reaction flasks of 140 ml volume and samples of 2 to 50 ml. Jaegers and Niemitz (1952) used the Warburg apparatus with 125 ml flasks specially designed to have a low hydrodynamic resistance, thereby reducing the strain on the manometer tubing.

Nelson _et_ _al_. (1961) used a Warburg apparatus with 125 ml flask for studying biodegradation of alkylbenzene sulfonates. The reaction volume was 20 ml, and included concentrated activated sludge and alkylbenzene sulfonate (ABS) solutions. Oxygen uptake was measured over a period of 28 hours. Buzzell _et_ _al_. (1969) used similar flasks for studying the biodegradability of several families of organic chemicals. Oxygen consumption was measured up to a period of 12 hours. At the end of the run, these researchers also determined the soluble carbon remaining in the Warburg flask to support the oxygen consumption data and to confirm the completeness of the degradation process. Further, to obtain a complete picture of the behavior of the organic chemical, Buzzell _et_ _al_. (1969) carried out parallel shake culture studies, (see Section III A.3, p. 83) in order to determine changes in total organic carbon, and used a dehydrogenase enzyme assay to follow the changes in the metabolic activity of the biological agent.

Barbaro and Hunter (1965) utilized the Warburg apparatus equipped with flasks of 140 ml capacity to study the effect of clay minerals on surfactant biodegradability. Use of a large flask permitted them to increase the sample volume to 50 ml and therefore data on oxygen uptake could be collected for up to 10 days.

(iii) Differential Manometer

Dick (1964) has reported that the accuracy of the Warburg test is low for relatively small O_2 uptake rates in waste waters. Schulze and Hoogerhyde (1967), therefore, tested the utility of the differential manometer for BOD measurements. A modification of the instrument developed by Schulze and Hoogerhyde (1967) is shown in Figure 6. The unit uses a 300 ml flat bottom sample flask and a 300 ml flat bottom reference flask connected by a ground-glass joint to a capillary glass tube, which is connected to a manometer by two ground ball-joints. The flasks are submerged in a constant temperature water bath and paraffin oil is used as manometer fluid. The sample is agitated by a 1-inch long Teflon-coated magnetic stirring bar. The sample flask is also equipped with an 1/2-inch diameter hanging glass cup to which is added 10% KOH solution to absorb CO_2. The oxygen consumed is measured by a micrometer syringe. The measuring and sampling technique is described in detail by Hoogerhyde (1965).

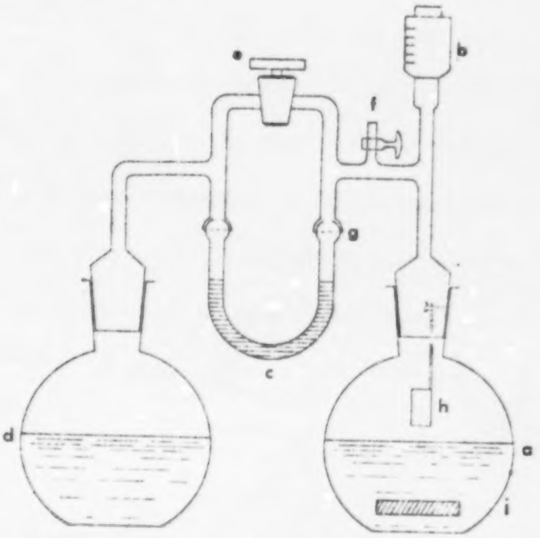

Figure 6. Diagram of differential manometer (Schulze & Hoogerhyde, 1967) a) sample flask, b) micrometric buret, c) manometer, d) reference flask, e) equilibration valve, f) valve to atmosphere, g) ball joints, h) KOH cup, i) magnetic stirring bar.

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(iv) Electrolytic respirometer

Liebammn and Offhaus (1966) have described a sapromat A6 respirometer, a patented electrolysis respirometer which is available from J.M. Voith, GmbH, Heidenheim, West Germany. Schematic diagram of the unit is shown in Figure 7. The oxygen pressure in this type of respirometer is automatically maintained at a constant value by an electrolysis cell, for the duration of the experiment. The current passing through the cell, which is proportional to the oxygen consumed by the sample, may be integrated and/or continuously recorded. The apparatus operates independently of the changes in barometric pressure. The whole apparatus is placed inside an incubator to minimize the changes due to temperature variations. The sapromat test is usually run for a period of 120 hours.

Pauli and Franke (1971) have used the sapromat apparatus to evaluate the effect of preservatives and disinfectants on the biological degradation of a standardized sewage. The test was run for 120 hours with intermediate samples drawn at 24 and 48 hours. The degradation obtained without the test chemical was expressed as 100%. A sapromat value higher than 100% was considered to be due to the degradation of the test chemicals.

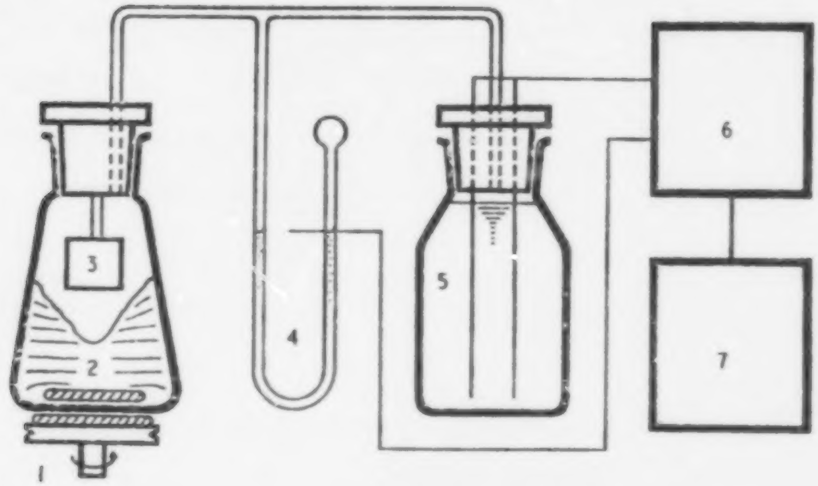

Figure 7. Schematic diagram of one of the six units of the Sapromat A6 Respirometer (Pauli and Franke, 1971).
1) magnetic stirrer; 2) sample; 3) CO_2 absorber; 4) manometer; 5) electrolytic cell; 6) measuring and control unit; 7) printer (optional). Reprinted from Biodeterioration of Materials published by Applied Science Publishers Ltd.

(v) Oxygen electrode respirometer

Oxygen electrode respirometers measure dissolved oxygen in the liquid phase, unlike the manometric method in which pressure changes in the gas phase are measured. This method allows an investigator to record continually the oxygen depletion which is related to the oxidation of the organic chemical. Although an oxygen-sensitive electrode can be used as an analytical tool to measure dissolved oxygen in 5-day dilution BOD test (APHA 1971,), the respirometric methods described in this section are somewhat different than the dilution method. First, the concentration of microorganisms in oxygen electrode respirometer test is generally higher and the test is run for a much shorter period than the dilution method. Second, the oxygen electrode respirometer has the reaction vessel linked to the metering device and this permits continuous measurement of oxygen uptake.

(a) Galvanic cell oxygen electrode respirometer

The assembly described by Gannon *et al*. (1965) consists of a standard two-liter sealed reaction flask with a specially constructed galvanic cell oxygen electrode (Gilman Instrument Co.) as developed by Mancy *et al*. (1963). The oxygen electrode is connected to a standard strip chart recorder to permit continuous recording of residual oxygen concentration with time. When the oxygen concentration in the flask drops to 2 mg/ℓ, the flask is aerated by means of compressed air and the procedure is repeated.

The oxygen analyzer consists of a silver-lead Galvanic couple separated from the test sample by a polyethylene membrane with a 1 M KOH solution between the couple and the membrane. One modification of the analyzer is the substitution of 1% methylcellulose (Dow Chemical Co.) in place of the lens paper as a carrier for the 1 M KOH to increase the sensitivity of the electrode.

This procedure has the same advantages as the reaeration technique in that no dilution is involved but has an added advantage of keeping the original sample intact, rather than splitting it up into several sealed BOD bottles.

(b) Clark-type oxygen electrode

This is a gold-silver electrode system which is protected by a thin gas permeable Teflon membrane (Clark *et al.*, 1953). The membrane isolates the sensor element from its environment, except that oxygen can diffuse into the sensor.

The sensor is 25 times more sensitive compared to conventional gas phase manometric systems (Yellow Spring Instrument Co., 1970).

Hammond and Alexander (1972) and Dias and Alexander (1971) have used Clark-type polarographic electrodes for BOD determination; the electrode was mounted in a rubber stopper so as to fit the neck of the BOD bottle. The advantages of using oxygen sensor over chemical methods in BOD determinations have been discussed by Reynolds (1969).

The sample volume in this assembly is restricted to 3 ml and, therefore, the amount of oxygen available is limited. The problem can be overcome by using a Macro bath assembly which allows an investigator to use sample sizes between 20-50 ml. This assembly may be more suitable in degradation studies where increased quantities of oxygen are desirable.

(c) Other Oxygen Electrode respirometers

Eye *et al.* (1961) used a solid oxygen electrode connected to a pH meter for BOD measurements. Samples were diluted as in the dilution test and dissolved oxygen was continuously measured by the oxygen electrode.

The apparatus developed by Lamb *et al.* (1964) is shown in Figure 8. This is similar to other systems using oxygen electrodes except that a diffuser is provided for aeration of the respirometer contents.

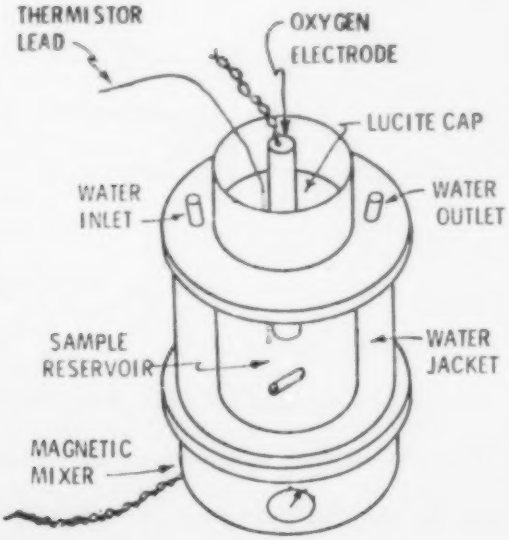
THERMISTOR LEAD
OXYGEN ELECTRODE
LUCITE CAP
WATER INLET
WATER OUTLET
SAMPLE RESERVOIR
WATER JACKET
MAGNETIC MIXER

Figure 8. Completely filled electrode respirometer (Lamb *et al.*, 1964) Reprinted with permission from Journal Water Pollution Control Federation, 36, 1263, Wash., D.C.

(vi) Miscellaneous techniques

A complete description of all the respirometric methods is available in the review by Montgomery (1967). Some brief descriptions of some of the other respirometry techniques will be discussed in this section.

The apparatus described by Popel *et al*. (1958) consists of a vertical reaction column connected to a manometer and a gas burette; at the bottom of the column is a gas diffuser. Air is continuously pumped in closed circuit through the column and through an external gas-washing bottle containing alkali, by means of a diaphragm pump. Excessive foaming was a problem with the apparatus when samples containing detergents were examined.

An automated apparatus is described by Snaddon and Jenkins (1964) in which instead of noting the change in manometer reading, a measured volume of oxygen is added to balance the manometer, automatically by a system of pipettes and electromagnetic valves. A modified automatic respirometer is described by Wilson (1967) which has a provision for circulating the gas phase continuously through an alkali scrubber.

Busch *et al*. (1962) developed a technique to study biodegradability by determining the theoretical oxygen demand (BOD corresponding to exhaustion of the substrate plus the oxygen equivalent of the new cells formed). Sewage was allowed to settle for 24 hours, filtered, and then served as the seed in this test. The BOD measurements were carried out by standard methods. Cell yield was determined by weighing the cells after filteration. Oxygen equivalents were determined by assuming that the general formula for the cell contents was $C_5H_7NO_2$. It has been suggested that the assumed formula might not always be correct and therefore error could be introduced (Grady and Busch, 1963).

2. River Die-Away Test

The river die-away test is a static type biological system and has been extensively used in biodegradation studies. This type of test system is an attempt to simulate biodegradation conditions of a river, lake, lagoon, or marine type environment. In this test, raw water is collected from a river and allowed to stand for a day and the water is decanted to remove large particles and mud. The chemical compound of interest is added to the river water contained in a glass jar and the solution is analyzed at various intervals to determine degradation by one of a variety of analytical techniques. The microbial content of the river water is normally low, although the number could vary significantly depending on the site chosen. Rivers receiving domestic and industrial waste are likely to have a much greater microbial population. The size of the water sample is largely dependent on the analytical requirements. In studies reported, the sample size has varied anywhere between 1-20 liters. The type of containers used include mason jars, glass bottles, etc. The period of incubation of the test chemical with river water normally ranges from a few days to up to 8 weeks, depending on the observed rates of biodegradation. This period includes the time required for acclimation of the river bacteria, which varies from compound to compound.

a. Original River Die-Away Test

The river die-away test has been extensively used in studying biodegradation of surfactants. In these studies, various sources of river water and test chemical concentrations have been used. A summary of a number of river die-away tests is given in Table 5. Selected other studies are discussed below in greater depth.

The river die-away test orginially described by Hammerton (1955) was employed to study the degradation of sodium lauryl sulfate at 4 ppm. The period of standing ranged from 18-36 hours. When the depleted solution was redosed with detergent, its concentration was reduced even more rapidly than the first time. Degradation was also examined when water was boiled or phenylmercuric acetate was added to inhibit bacterial action, or when detergent was dissolved in distilled water. In a modified river die-away test, Borstlap and Kooijman (1963) used distilled water supplemented with a small quantity of activated sludge, instead of river water. Lashen et al. (1966) carried out river die-away tests with nonionic-surfactant alkylphenol ethoxylate in the water samples collected from several locations on a number of different rivers.

Table 5. River Die-Away Test for Determining Biodegradability of Organic Chemicals

Reference	Compound Tested	Concn.	Source of River Water
Sawyer *et al.* (1956)	Detergents	5 ppm	Two sources in Massachusetts
Blackenship and Piccolini (1963)	Nonionic detergents	20 ppm	Delaware River
Weil and Stirton (1963)	30 anionic and 6 nonionic detergents	-	Schuykill, Susquehanna River and Delaware River
Setzkorn *et al.* (1964)	Detergents	10 ppm	Mississippi, Missouri, Ohio, Wabash, Kansas and Arkansas Rivers
Weaver and Couglin (1964)	Surfactants	20 ppm	Ohio River
Bacon (1966)	Surfactants	7 ppm	Detroit River at the intake to City Water Plant
Osburn and Benedict (1966)	Polyethoxylated alkyl phenol	200 ppm	Ohio River
Swisher (1967a)	Surfactants	5 mgs	-
Warren and Malec (1972)	Nitrilotriacetic acid and related imino and amino acids singly or in combination.	20 ppm	Detroit and Meramec Rivers
Swisher and Gledhill (1973)	o-benzyl-p-chlorophenol (Santophen I)	0.1 mg/ℓ	Meramec River

Sample sites included highly industrialized areas where microorganisms may be expected to be acclimatized, rural suburban areas which probably had some exposure to nonionics, and representative water from non-industrialized area having little or no exposure to nonionic surfactants.

Only a few reports are available on studies concerning biodegradation of pesticides in river water. Eichelberger and Lichtenberg (1971) have studied the persistence of 28 common pesticides in river water from the Little Miami River over a period of 8 weeks. The concentration of pesticide in this study was initially 10 mg/ℓ. The concentration is somewhat higher than usually found in surface waters, but was used to facilitate the identification of decomposition products. The mixture was incubated in sealed glass jars on a laboratory bench under simulated sunlight. The contents were shaken periodically to redistribute the pesticide and any suspended matter that might have settled out. Identification of chemical degradation was made by running a parallel experiment in which distilled water was used as a replacement for river water.

Biodegradation of urea in river waters under winter conditions was studied by Evans et al. (1973). Winter conditions were chosen since urea, among other things, is used as a deicing agent. Samples of Yorkshire river water (England) of varying composition were obtained and bottom mud was added. The concentration of urea was kept low, between 1-15 mg/liter. Evans and David (1974) in the biodegradability studies of glycols used river waters of varying composition and topographic origin obtained from 4 different sources. Studies were carried out at temperatures ranging from 4-20°C.

b. River Die-Away with Fortified and Inoculated Waters

Since the composition of the river water chosen for the river die-away tests varies considerably in microbiological and chemical terms, the reproducibility of the biodegradation results has been poor (Swisher, 1970). Furthermore, in view of the fact that the concentration of microorganisms and essential nutrients required for their growth is generally low in river water, the time of incubation of the test chemical with the river water has been long. To overcome these difficulties, attempts have been made to devise fortified waters for use in this test.

Garrison and Matson (1964) inoculated river water with activated sludge from a predominantly domestic source. The sludge was filtered and added to a concentration of 0.1% (w/v). Conway and Waggy (1966) in their test have used half-gallon bottles charged with one liter of river water containing 0.5% of settled sewage. Surfactant concentration was 10 mg/ℓ. The bottles were loosely capped during the test. Two bottles, to which surfactants were not added, served as controls. Eden _et_ _al_. (1967) incubated for periods up to three weeks, a dilute solution of the detergent material in river water seeded with a small quantity of sewage effluents. The test was conducted in open or closed bottles in a manner similar to the BOD test (see Section III A.1,a.i, p.52). Since a limited amount of oxygen is available in the closed bottle test, both the amount of inoculum and the initial concentration of surface active material is limited.

In a new accelerated biodegradation test (Hitzman, 1964), the procedure employed was the same as for the river die-away test described by Proctor and Gamble (1964) except that the natural water was fortified with microorganisms obtained by continuous centrifugation of water from a municipal sewage plant effluent or river water itself. In order to achieve reproducibility between the results obtained by different laboratories, the fortified water was diluted to a standard optical density so that the number of cells in each test was somewhat similar. The French IRChA tecnhique (Institut National de Recherche Chimique Appliqiue) (Brebion et al., 1966) uses river water medium fortified with additional microorganisms from a culture developed from sewage-polluted river water. Nutrients are added in the form of meat extract and peptone to sewage-polluted river water and microorganisms are allowed to multiply. For the test, unpolluted river water containing surfactant (10 ppm) was inoculated with 10-20% of the above culture (bacterial count between 200 and 400 million/ml). The test mixture was redosed with surfactant on the seventh day.

A technique to demonstrate acclimation of river microflora to alkylphenol ethoxylate has been described by Lashen and Booman (1967) and is based on the presumption that acclimated bacteria, when diluted greatly in unacclimated river water solution, should initiate an immediate degradation when the test compound is biodegradable. In this procedure, a fresh uninoculated Delaware river water sample was inoculated with 1% of the acclimated river water. A portion of the Delaware river water was left uninoculated to serve as a control.

To study the biodegradability of the organic pesticides, Sevin, 1-naphthol, baygon, pyrolan and dimetilan, Aly and El-Dib (1972) have used Nile River water buffered with phosphate to maintain a pH of 7.2 ± 0.1. Insecticides were added each at a concentration of 4 mg/liter and 10 liter portions of each solution placed in a 5 gallon container. The incubation was carried out at room temperature and aerobic conditions were maintained by bubbling a gentle stream of air. The disappearance of the compound was also followed after redosing with insecticide, to check for possible acclimation. If an insecticide was found to be degrading slowly, river water was supplemented with 1% of settled sewage to increase the rate of degradation.

Biodegradation of sulfonated amide surfactantswas studied by Sheers et al. (1967) using the water from the North Branch of the Raritan River at a point which had a minimum possibility of contamination by detergents. The water, following filtration through cotton, was distributed in 16-liter portions in 20-liter bottles. Inorganic nutrients were added to furnish the necessary P and N concentration based on the COD values of each bottle. Test material (5 and 10 mg/ℓ) was added and the bottles were aerated.

Horvath (1972b) modified the river die-away test to study cometabolic degradation. A biodegradable analogue of the test chemical was added along with the test chemical. In studies on the degradation of trichlorobenzoate, the biodegradable analogue used was sodium benzoate, which was added at twice the concentration of the test chemical.

c. River Die-Away Test with Polluted River Water

In several biodegradation studies, polluted water collected from rivers receiving domestic and industrial waste has been used because such waters contain greater microbial populations and often contain certain specialized microorganisms. The source of bacteria utilized by Wayman and Robertson (1963) in the degradation of anionic and nonanionic surfactants was South Platte River water sampled near the discharge pipe of the Denver sewage treatment plant effluent. The chemicals discharged into this water over a three-year period included ABS in the range of 2-8 ppm. Biodegradation was studied in solutions prepared by dilution of river water by as much as 100 times with distilled water or by using the river water undiluted. The surfactant concentration was 25 ppm. The stability of detergents was studied under aerobic, as well as anaerobic bottles. Inorganic nutrients were added to furnish the necessary P and N concentration based on the COD values of each bottle. Test material (5 and 10 mg/ℓ) was added and the bottles were aerated.

Horvath (1972b) modified the river die-away test to study cometabolic degradation. A biodegradable analogue of the test chemical was added along with the test chemical. In studies on the degradation of trichlorobenzoate, the biodegradable analogue used was sodium benzoate, which was added at twice the concentration of the test chemical.

c. River Die-Away Test with Polluted River Water

In several biodegradation studies, polluted water collected from rivers receiving domestic and industrial waste has been used because such waters contain greater microbial populations and often contain certain specialized microorganisms. The source of bacteria utilized by Wayman and Robertson (1963) in the degradation of anionic and nonanionic surfactants was South Platte River water sampled near the discharge pipe of the Denver sewage treatment plant effluent. The chemicals discharged into this water over a three-year period included ABS in the range of 2-8 ppm. Biodegradation was studied in solutions prepared by dilution of river water by as much as 100 times with distilled water or by using the river water undiluted. The surfactant concentration was 25 ppm. The stability of detergents was studied under aerobic, as well as anaerobic conditions. Aerobic systems were studied by continuously bubbling filtered air through solutions. Anaerobic systems were studied by use of a Brewer anaerobic jar or without bubbling air through the surfactant solutions (the later system need not necessarily be anaerobic). Various temperatures ranging from 10 to 35°C were employed in the study.

Ettinger and Ruchhoft (1950) have examined the fate of monochlorophenols in polluted river water obtained from the Great Miami River and the Little Miami River. In their studies, the compound was attacked more readily when added to river water than when added to diluted sewage. The more rapid attack was attributed to the presence of microorganisms in the river water capable of destroying ortho- or para-chlorophenol. Persistence of pyridine bases at a concentration of 1 ppm using polluted river water from several different sources - Ohio River water sampled both above and below Cincinnati; Great Miami River water taken near the mouth - was examined by Ettinger et al. (1954). With the exception of Little Miami water, all the river waters were known to have a history of pollution by waste containing pyridine bases. In an initial persistence experiment, these investigators also studied the degradation of pyridine in tap water containing 1% dilutions of Cincinnati sewage.

Yasuno et al. (1966) studied the degradation of organophosphorus insecticides in polluted water. As a source of polluted water, these investigators used (1) sewage water obtained from a natural breeding place of the test insect (Culve pipiens molestus) used for the biological assay, and (2) artificially prepared polluted water (3 gm of unsterilized laboratory animal food powder added to one liter of tap water and kept for 7 days at 28°C). The concentration of insecticide added to pure and polluted water started from 80 ppm, and twofold dilutions were made such as 40 ppm, 20 ppm, 8 ppm, 4 ppm, 2 ppm, 0.8 ppm, up to the lowest of 0.0008 ppm for some insecticides. The insecticidal activity of the medium was tested at 1, 4, 8 and 16 days.

d. Anaerobic and Microaerophilic River Die-Away Test

Degradation of several anionic detergents under anaerobic and microaerophilic conditions has been studied by Maurer et al. (1971) using river water as the source of inoculum. Detergent solution of 5 or 10 ppm prepared in Schuylkill River water was transferred in 2 liter portions into vented 3 liter aspirator bottles. The bottles were equipped with extracourse spargers through which nitrogen gas or air was passed (Figure 9). Bottles had been wrapped with foil to minimize the growth of algae which may release oxygen into the system.

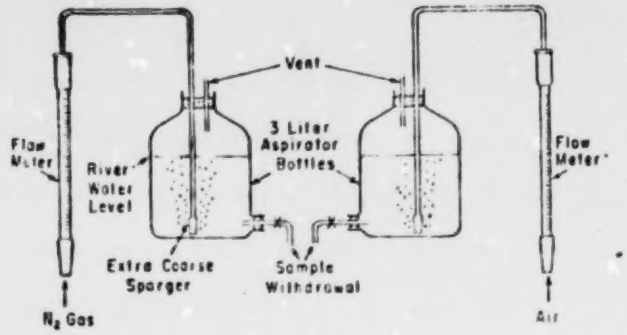
Vent
Flow Meter
River Water Level
3 Liter Aspirator Bottles
Flow Meter
Extra Coarse Sparger
Sample Withdrawal
N_2 Gas
Air

Figure 9. Experimental apparatus used for simultaneous microaerophilic and aerobic tests (Maurer et al., 1971) Courtesy of J. of the Amer. Oil Chemists' Society.

The anaerobic die-away test used by Conway and Waggy (1966) is simply a moderately seeded river die-away test conducted under anaerobic conditions. Surfactant was dissolved in oxygen-free river water which was seeded lightly with sewage. The solution was purged with nitrogen to remove dissolved oxygen. The bottles were sealed, inverted and stored in dark. Aerobic die-away tests were concurrently run for comparison.

e. Die-Away Test with Marine Water

Recently, efforts have been extended to the study of compounds using sea water as inoculum. In the test described by Atlas and Bartha (1972, 1973), degradation of petroleum was studied in the sea water collected off the east shore of Sandy Hook, N.J. Sea water samples were supplemented with KNO_3 and Na_2HPO_4 as nitrogen and phosphorus sources, respectively. One-hundred-milliliter portions of the sea water were introduced into the flask component of the gas train assembly for CO_2 evolution measurements. Filter sterilized crude oil was added to the sea water as 800 mg fresh or 560 mg "weathered oil" and the flasks were continuously aerated with CO_2-free air. Since the temperature in the ocean is lower than optimum for many microorganisms, these investigators maintained the temperature between 5 and 20°C.

3. Shake Culture Test

In this technique a pure culture, or a mixture of microorganisms from natural sources such as soil, water, activated sludge, sewage, etc. or from active mixed laboratory cultures are grown and/or adapted in a medium containing the organic compound to be tested. The medium used for this type of test generally contains balanced nutrients and essential mineral elements. The flasks are aerated and at certain intervals aliquots are withdrawn to test for biodegradation. Based on the type of inoculum used and whether the microorganisms used for biodegradation have been adapted, the shake flask culture test can be run in several ways.

a. Shake Cultures Inoculated with Natural Communities of Microorganisms

(1) Degradation tests using activated sludge or sewage as source of microorganisms

(a) Original shake culture method for study of surfactant biodegradation.

Huddleston and Allred (1963) have described a shake culture test in which they employed a mixed culture obtained from an activated-sludge-type waste-treatment plant as the source of inoculum. The culture was adapted by three successive transfers in a medium (composition shown in Table 6) containing 30 mg/liter detergent. The adapted seed (10 ml) was finally used to inoculate sterilized 1 liter medium containing 30 mg of test detergent in a 2 liter Erlenmeyer flask. The culture was incubated at 25°C on a rotary shaker. Samples were withdrawn at desired intervals for analysis. The test has been used extensively to study the biodegradability of surfactants (Allred _et_ _al_., 1964; Huddleston and Allred, 1965).

Table 6.

Composition of the basal medium (Huddleston and Allred, 1963)

$NH_4C\ell$	3.00 g
K_2HPO_4	1.00 g
$MgSO_4 \cdot 7H_2O$	0.25 g
KCl	0.25 g
$FeSO_4$	Trace
Yeast extract	0.30
Distilled water	1.0 liter
pH after sterilization	7.1
Detergent	0.030 g

Setzkorn and Huddleston (1965) in a modified test used reduced concentrations of inorganic salts and yeast extract (Table 7). This was done to facilitate the use of U.V. analysis (described in detail in Section III C.2.d., p.156) to follow the rupture of the benzene ring system. The incubation was performed in 1/2 gallon fruit jars which contained 5 ppm of the test compound and 5 ml of raw sewage in 1 liter of synthetic medium.

Table 7.

Composition of Medium (Setzkorn and Huddleston, 1965)

Compound	Wt. g
NH_4Cl	0.1500
K_2HPO_2	0.0750
$MgSO_4$	0.0125
KCl	0.0125
Yeast extract	0.0050
Deionized water	1.0 liter

(b) Shake culture test of the Soap and Detergent Association (SDA)

The subcommittee on biodegradation test methods of the Soap and Detergent Association has adopted, with minor modifications, the shake flask method described by Huddleston and Allred (1963) to be used as a screening or presumptive test (SDA, 1965). If the degradation in the presumptive test equals or exceeds 90%, the compound is considered adequately biodegradable by SDA. If, however, degradation falls between 80 and 90%, the biodegradability is confirmed by semicontinuous activated sludge procedure (see Section III B.2.b, p.139). The medium used in the shake culture test is the same as described by Huddleston and Allred (1963) (see Table 6). The microbial inoculum for the test may be one of the following:

1. Natural Sources (soil, water, sewage, activated sludge, etc.)
2. Laboratory culture (activated sludge, river die-away, etc.)
3. Culture obtained from:

   Laberco Laboratories, Inc.
   123 Hawthorne Street
   Roselle Park, N.J. 07204

The seed is adapted by making two 72-hour transfers. For the final inoculation 1 ml culture is used for each 100 ml of fresh medium and the test is run for 8 days.

(c) Bunch and Chambers Test

Bunch and Chambers (1967) described a shake culture test in which they used BOD dilution water supplemented with yeast extract as the basal medium. Their preference for BOD water over the nutrient medium of Huddleston and Allred (1963) was due to the fact that BOD dilution water is already available in many laboratories that would be using a test for biodegradation. In this test 10 ml of settled sewage is used to inoculate 90 ml of BOD dilution water containing 5 mg of yeast extract and a suitable amount of test compound (approximately 2 mg/100 ml). The test is run in 250 ml Erlenmeyer flasks which are aerated on a shaker at ambient temperature 25°C ± 5°. Weekly subcultures are made in fresh medium for three consecutive weeks (total time of the test is 28 days). The flasks are examined the day following inoculation and if no turbidity is found then the test is repeated with the same and three lower concentrations of the test compound.

Leigh (1969) modified the Bunch and Chambers test in order to study the degradation of compounds such as chlorinated hydrocarbons, which have low solubility in water. Aqueous solutions of the insecticides (saturated) were prepared in distilled, deionized and charcoal-filtered water. Inorganic salts and yeast extract were added to the insecticide solution as in the preparation of BOD water. The mixture was seeded prior to distribution into the Erlenmeyer flasks.

(d) Shake culture test utilizing preserved seed

The variations in the type of inoculum used by different laboratories for testing biodegradability has sometimes made it difficult to make comparisons between the results. For this reason attempts have been made to use a seed which could be readily duplicated in other laboratories or conveniently stored. This includes means of preserving the seed in the dormant form which could be rendered viable at the time of use.

(1) Air-dried activated sludge - The Aeration Test

Truesdale et al. (1969) have described a test referred to as the "aeration test" which involves incubation of a dilute solution of the detergent in standard BOD dilution water under aerobic conditions with air-dried activated sludge as the inoculum. This test was adopted by British Standing Technical Committee on Synthetic Detergents (British STCSD) for testing biodegradability of detergents. The test is also referred to as the British STCSD Test (Swisher, 1970). Air-dried activated sludge was prepared by evaporating to dryness a thin layer of sludge at room temperature. Dried sludge retained its activity for many weeks without special conditions for storage. These investigators discarded freeze drying as a method of preservation since it required the addition of a preservative which could complicate the results.

The test was used for studying the biodegradability of synthetic detergents (Eden et al., 1967; Truesdale et al., 1969). The detergent concentration was kept at 10 mg/liter of BOD water. The mixture was inoculated with 30 mg/liter of dried sludge and aerated by gentle stirring. Measurements were made for a period of 12-21 days.

Patterson et al. (1967, 1968) used the aeration test, after introducing a few variations, for studying the biodegradation of alkyl phenol polyethoxylates. In order to remove visible dried sludge particles from the solution, which may adsorb the detergent, these investigators filtered the mixture through a Whatman No. 1 filter paper, after overnight aeration in BOD water. Furthermore, whenever the detergent concentration was increased, the proportion of dried activated sludge was also increased to accelerate the degradation.

In order to avoid lengthy acclimation periods for compounds which degrade very slowly, Truesdale et al. (1969) modified the aeration test by using an acclimated microbial culture which was obtained from a recirculation filter. The presence of active inoculum from this source increased the rate of degradation considerably. However, the authors commented that with this inoculum, the degradation observed was considerably greater than achieved in normal treatment plant practice.

(2) Shake culture test using sludge preserved by lyophilization

Buzzell et al. (1969) have investigated and evaluated various methods for preserving standardized activated sludge.

The sludge was prepared in a Bench Scale continuous-flow model activated sludge plant (see Sec. III B.2a, p.129), which was inoculated with activated sludge obtained from a municipal treatment plant receiving sewage largely of domestic origin. In order to provide a source of nitrogen and phosphorus, a synthetic sewage feed consisting of glucose and peptone and inorganic salts dissolved in tap water was used with the model activated sludge plant (Wiener, 1966). Activated sludge, acclimated to the synthetic sewage was preserved using the following procedures:

1. Freezing slowly at -15°C (deep freezer unit).
2. Freezing quickly at -76°C (acetone and dry ice).
3. Freezing quickly at -192°C (liquid nitrogen).
4. Lyophilization

The activity of the sludge preserved by the procedures described above was evaluated by measuring oxygen uptake in a Warburg respirometer with glucose plus peptone as substrate. The results indicated (Figure 10) that the lyophilized sludge was the most vigorous since it used oxygen at significantly greater rates than sludges preserved by other methods. Consequently lyophilization was preferred.

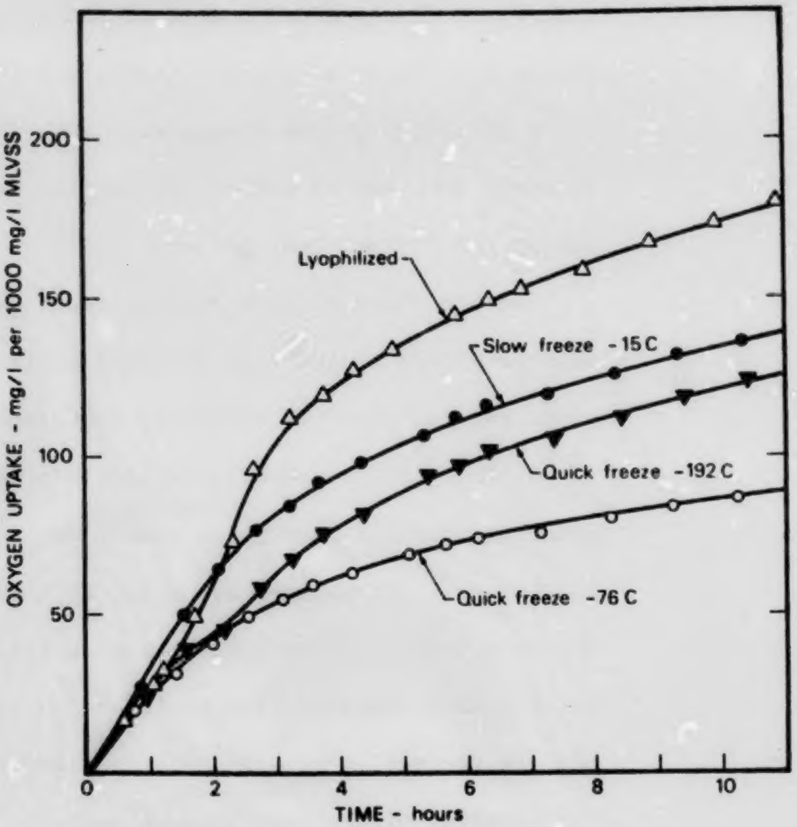

Figure 10. Oxygen Uptake by Sludges Preserved by Four Methods. Substrate: Glucose plus Peptone (Buzzell et al., 1969) Courtesy of Manufacturing Chemists Association.

In order to reactivate the dried sludge for use in shake culture studies, it was mixed with synthetic sewage. The rejuvenation process was carried out in batch units which were fed at 12-hour intervals. A rejuvenation period of 24 hours was found to be optimum since it gave rise to microorganisms with the highest respiration rates.

Shake culture studies were carried out in 500 ml wide-mouth flasks which contained 100 ml of reactivated sludge and 100 ml of organic chemical solution (180 mg/liter as carbon). The flasks were covered loosely with aluminum foil and shaken at 120 oscillations per minute during the 12-hour test period.

In the shake culture studies described above, the adverse effect of the test material on the biological agent was monitored by measuring dehydrogenase activity, an enzyme system responsible for oxidizing or dehydrogenating unspecified organic compounds in the cell. Furthermore, the pattern of oxygen utilization was also observed using a Warburg respirometer (see p. 61). The results were combined with the shake culture studies and all the derived information was considered for evaluating the behavior of the organic chemical.

(e) Degradation methods utilizing composite seed

Sturm (1973) suggested a biodegradability screening test which used a composite seed prepared by mixing equal volumes of cultures, each of which had been acclimated to a test compound. The advantages of common composite seed are: (1) it allows using a single blank for many test units, and (2) it provides a bacterial population acclimated to a variety of chemicals. Using this procedure these investigators studied the degradation of eight nonionic surfactants. Individual acclimation cultures for each chemical and a dextrose control were prepared in 2-liter capacity wide-mouth flasks, which contained settled raw sewage as a source of microorganisms, yeast extract as an easily utilizable nutrient source, BOD water as a diluent and source of inorganic nutrients, and a test material; the mixture was incubated for 14 days in the dark. Equal aliquots from each of these cultures were used to make a composite seed. A 10% solution of the composite seed in BOD water was prepared and aerated for 24 hours. Subsequently, test material (20 mg/liter) was added and degradation was studied (see Figure 11).

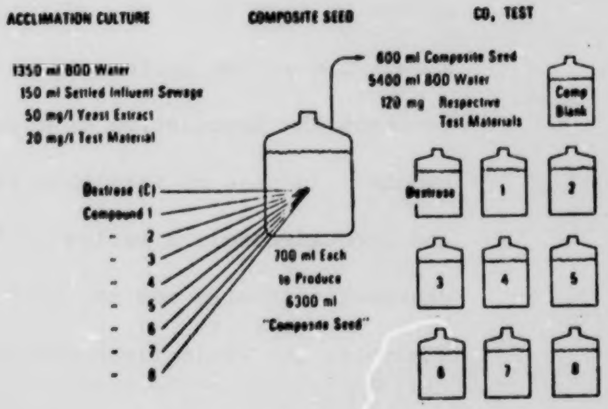
ACCLIMATION CULTURE
COMPOSITE SEED
CO$_2$ TEST
1350 ml BOD Water
150 ml Settled Influent Sewage
50 mg/l Yeast Extract
20 mg/l Test Material
Dextrose (C)
Compound 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
700 ml Each
to Produce
6300 ml
"Composite Seed"
600 ml Composite Seed
5400 ml BOD Water
120 mg Respective
Test Materials
Comp
Blank
Dextrose
1
2
3
4
5
6
7
8

Figure 11. General Protocol for a 10 Unit Biodegradation Test Unit (Sturm, 1973) Courtesy of J. of the Amer. Oil Chemists' Society

Microbial cultures used by Garrison and Matson (1964) in their degradation studies were obtained from the Soap and Detergent Association (SDA) which had adapted them to a medium containing 30 ppm of linear alkylbenzene sulfonate (LAS) prepared from dodecene-1. The above investigators modified the cultures by growing them in a SDA medium containing mixed detergent feed. The authors suggested that the adaptation procedures described above will develope cultures which can be used in studies with a wide variety of detergents.

For use as seed, Langley (1970) developed a heterogenous population by growing the microorganisms present in settled sewage supernatant on a mixture of primary alcohols (ethanol, 1-propanol, 1-butanol, and isobutanol). The cultures were maintained by daily removal of one-third of the culture fluid and addition of fresh alcohol mixture. The microorganisms adapted in this manner were used to study the degradation of higher molecular weight alcohols. In case of insoluble substrate, e.g., 1-hexadecanol and 1-octadecanol, a series of flasks, each containing identically treated medium, were used. For periodic analysis, the whole flask was sacrificed.

(f) Slope (Slant) culture technique

In this test, acclimated seed is prepared by inoculating an agar slant containing approximately 10 mg/liter of the test detergent with the mixed bacterial culture from sewage works effluent (Cook, 1968). Agar slants are incubated for 7 days and the bacterial growth is used to inoculate a detergent solution prepared in standard dilution water. Organisms suspended from two slants were used to inoculate one 500 ml of the detergent solution. Samples were analyzed for degradation prior to inoculation and for a further 15 days. In the initial studies, Cook (1968) used four different effluents having varying proportions of industrial waste, domestic sewage and agricultural waste as the source of inocula for the slant culture technique to see if the reproducibility of the results was affected. It was concluded that any effluent could be used as the source of inoculum without significantly affecting the results. Degradation was also studied using unacclimatized seed prepared by inoculating agar slants without the test detergent. Since the bacteria that grew on the slants not containing the detergents degraded the test detergents just as effectively as those which had been exposed to the test detergent, the author concluded that the bacteria had not been acclimated by slant culture.

In several tests Cook (1968) also used suspensions of organisms obtained from recirculating filters (see p. 146), as the source of inoculum for agar slopes. The microbial population developed in the form of visible slime (organisms grown on test detergent as the sole source of organic carbon) in the recirculating filters was transferred into Ringer solution in McCartney bottles and shaken. The suspension thus prepared was used to inoculate agar slants without added detergent.

Dobane 055, a synthetic anionic detergent, was not degraded by acclimated or unacclimated cultures obtained from the agar slants inoculated with sewage work effluent (Cook, 1968). However, when the inoculum prepared on agar slants from recirculating filter inoculum was used, the detergent was degraded after an initial lag. It was known that the bacteria were not acclimated by the slope culture technique and, therefore, the author speculated that the acclimation must have occurred during recirculation.

(g) Shake culture employing seed acclimated to increasing concentration of the test chemical.

Schwartz (1967) studied the degradation of pesticides utilizing microorganisms from an activated sludge unit and the effluent from a primary sedimentation basin of the Whittier Narrows Water Reclamation plant. In addition, samples of the flow and slime from a channel containing refinery waste effluents were used. The medium contained mineral salts (Gray and Thornton, 1928), various amounts of pesticides, supplemental organic carbon source and microorganisms. The breakdown was studied in a series (one to six) of adaptation stages; once significant or complete degradation became evident, transfer was made using the microbial inoculum from the preceding adaptation stage. During these stages the amount of pesticide was increased and the supplemental nutrient concentration was decreased.

Hemmett (1972), using a similar approach, studied the biodegradability of phenoxyacetic acid herbicides and phenols. Activated sludge, which was used as a source of aquatic microorganisms, was incubated in a mixture of synthetic sewage and an appropriate concentration of the herbicide. By addition on alternate days, the herbicide concentration was increased and the synthetic sewage concentration was decreased. At the end of the adaptation period (approximately 14 days) the cultures were used in degradation studies.

(h) Other modified tests

Thompson and Duthie (1968) prepared acclimated seed for studying breakdown of NTA by exposing microorganisms present in settled raw municipal waste water to nitrilotriacetic acid (NTA) in Bunch and Chamber (1967) medium. The adaptation was continued for 14 days. Degradation studies were performed in a 8-20 liter carboys which contained diluted seed (1:10 with BOD water) and NTA. The mixture was aerated with CO_2-free air.

Cordon et al. (1968) studied the biodegradability of anionic tallow-based detergents in 1-gallon wide-mouth jars which contained 3 liters of deionized water, 10 mg of activated sludge per liter (on dry weight basis), nutrient salts (free of sulfate to permit the assay of sulfate ion formation from anionic detergents) and 40 mg of detergent per liter. Activated sludge was obtained from a treatment plant which treats mostly domestic sewage. The detergent already present in the sludge was removed by deacclimation in a laboratory model activated sludge plant (Ludzack, 1960). A similar method was used (Cordon et al., 1972) to study the degradation of sulfonated alkanol amides. The test was run under aerobic and microaerophilic conditions. The microaerophilic test was carried out as described by Maurer et al. (1971) (see p. 81) except that instead of using river water, an inorganic nutrient salt solution was used as the medium.

Ferguson et al. (1973) studied the environmental fate of nitrilotriacetic acid (NTA) using four sources of organisms: a small stream, a farm pond, a river below Athens sewage effluent, and the pilot plant treatment plant at the Robert A. Taft Water Research Center, Cincinnati, Ohio (with prior exposure to NTA). Shake flasks contained 5 ml seed, basal salts (Payne and Feisal, 1963) and 10 mg/liter NTA. NTA degradation was also studied in the presence of an external carbon source; the investigators used glucose (10-100 mg/ℓ) instead of the more commonly used yeast extract. The flasks were incubated for 22 days and samples were withdrawn for analysis every 48 hours.

Pawlowski and Howell (1973a & b) used activated sludge and soil as the source of mixed culture of microorganisms. To maintain a constant inoculum for experiments, the inoculum was first acclimated at 28°C in a chemostat with a feed medium containing 100 mg/liter phenol. Two separate residence times in the continuous culture apparatus gave rise to two populations of microorganisms which were used in the degradation studies.

In his shake culture studies, Gledhill (1974) used a Bellco waffled Erlenmeyer flask which had been equipped to trap $^{14}CO_2$. Using this system he examined the breakdown of trichlorocarbanilide with raw sewage and activated sludge seed (from laboratory semi-continuous activated sludge unit) diluted with BOD water. For aeration, the flasks were sparged periodically with 70/30 O_2/N_2 mixture.

The activated sludge obtained from a local treatment plant was found to display an 8-10 weeks lag before acclimation was gained.

(ii) Degradation Test Using Lagoon Microorganisms

A method has been suggested by Halvorson et al. (1971) for testing the biodegradability of insecticides using resting cell suspensions of the bacteria from a sewage lagoon. The use of lagoon microorganisms was preferred because bacteria indigenous to this environment have been shown to exhibit a wide spectrum of physiological properties. The bacteria recovered from 40 liters of lagoon water were washed and resuspended in 200 ml of potassium phosphate buffer (pH 7.0) (about 4×10^8 cells/ml). Organophosphate insecticides were added at an initial concentration of 50 ppm. For studying biodegradation of chlorinated hydrocarbons lower cell populations and lower concentrations of the test chemical were used. Small aliquots of the reaction mixture were incubated in small vials. The entire contents of the vial were analyzed at regular intervals.

(iii) Shake culture test without initial inoculation

Considering the possibility that the cultures ordinarily used in biodegradation studies may be overspecialized and, hence, may give unrealistically good results, Swisher (1966) in his studies used the microorganisms which develop in an uninoculated test medium. This development of organisms from ambient sources is also used in the detergent continuous activated sludge test required by German law (German Government, 1962) (see Sec. III B.2.a, p.134). Swisher (1966) used the medium developed by Allred _et_ _al_. (1964) which was prepared and handled without aseptic technique. Surfactant was added to the medium contained in 1 liter Erlenmeyer flasks and then shaken on a rotary shaker. After one week, 1% of this mixture was inoculated into fresh medium plus surfactant and successive weekly transfers (for 4-5 weeks) were continued in this manner.

Swisher (1968) in his later studies on linear alkylbenzenesulfonate (LAS) degradation used the cultures which had developed in the uninoculated medium containing C_{12} LAS, and maintained through 61 weekly transfers on SDA medium. In much of the degradation work, Bunch and Chambers (1967) medium was used since the interference with the analytical test (U.V. analysis) was minimal. The transfers were made at biweekly intervals instead of weekly.

(iv) Shake cultures inoculated with lake sediments

Graetz _et_ _al_. (1970) have used the water obtained by centrifugation of Lake Tomahawk sediment as a source of microbial species. Sterilized 2% peptone solution was inoculated with the water obtained as described above; after 36 hours, when cloudiness had developed, the culture was diluted twofold with a 5 mg/liter parathion solution and degradation was studied. Degradation was also followed in a sediment system consisting of 90 mg Lake Tomahawk sediment and 200 ml of a 5 mg/liter parathion solution fortified with 5 ml of inoculated and incubated 2% peptone solution.

b. Shake Culture Studies Using Pure Cultures of Microorganisms

Shake culture studies using pure cultures of microorganisms have been extensively used in evaluating biodegradation. Biodegradability tests utilizing pure cultures will be expected to be more reproducible than if undefined mixed cultures are used as inoculum. A variety of sources of microorganisms have been used to obtain the pure cultures. These include pure cultures obtained from commercial sources or from a laboratory, pure cultures isolated from natural sources (without exposure to the test chemical), and pure cultures enriched from the natural population. In this method, sterile mineral salts culture medium (or salts medium supplemented with easily utilizable carbon source) is prepared and the organic compound of interest is included as the major potential nutrient. The medium is inoculated with an appropriate culture of microorganisms and aliquots are removed at various intervals for analysis.

Pure cultures are generally not considered as satisfactory inocula for preliminary screening. It is unlikely that a single species could be found which will be capable of assimilating all the chemicals, since the number of necessary constitutive and inducible enzyme systems capable of degrading organic compounds is limited in any single culture test system. By using a large number of pure cultures this problem can be partially overcome. When a large number is employed, the relative degree of biodegradability of a compound can be assessed from the proportion of the tested species which can utilize the compound as sole carbon and energy source (McKenna and Kallio, 1964; Painter, 1973a). In addition, pure culture studies should be of value in the detailed study of specific metabolic reactions.

On the basis of the sources used for procuring pure cultures of microorganisms for biodegradation studies, these studies could be grouped as follows:

(i) Pure culture obtained from commercial sources or from research laboratories

A number of pure cultures are generally tested for their ability to degrade the test compound. The organisms selected may be those which have been reported to metabolize certain synthetic organic compounds. Payne & coworkers (1970) and Painter (1973a) have suggested the use of species of genera such as *Klebsiella*, *Escherichia*, *Serratia*, *Candida*, *Pseudomonas*, *Flavobacterium*, *Achromobacter*, and non-parasitic species of *Mycobacterium* and *Nocardia*. Filamentous fungi such as members of the genus *Aspergillus* could also be added to the list since these organisms have been found to grow on a variety of substances (Murray *et al*., 1970).

A summary of the studies in which investigators have used pure cultures of microorganisms obtained from commercial sources or research laboratories is given in Table 8.

Table 8 Pure Culture Obtained from Commercial Sources or Research Laboratories

Reference	Organism	Source	Test Chemical	Conc.	Time of Incubation	External Carbon Source
Huddleston & Allred (1963)	*Escherichia coli*[a], *Serratia marcensens*[a], *Proteus vulgaris*[a], *Pseudomonas fluorescens*[a]		Sulfonated alkylbenzenes	30 mg/ℓ	3 days	None
Klug & Markovetz (1967)	110 species of *Candida*	1. North Regional Research Lab., Peoria, Ill. 2. Dept. of Microbiology, Univ. of Iowa 3. Amer. Type Culture Collection (ATCC) 4. Dr. Phaff, Univ. of Calif., Davis 5. Dr. Azoulay, France	N-alkanes, even-numbered 1-alkenes	0.2ml/ 20 ml	2 weeks	yeast nitrogen base
Goodnow & Harrison (1972)	19 Genera, 34 species and 45 strains of Bacteria, e.g. *Acetobacter*, *Azotobacter*, *Bacillus*, *Chromobacterium*, *Escherichia*, etc.	ATCC Dr. Engley Univ. of Missouri Medical School	Surfactants	0.004-0.5 g/ℓ	3 days	Trypticase soy broth without glucose
VanAlfen & Kosuge (1974)	*E. coli* *Pseudomonas cepacia*	Dr. Clark, Univ. of California, Berkeley Dr. Lorbeer, Cornell Univ.	2,6-Dichloro-4-nitroaniline	2 μg/ml	2 days	None

[a] Adapted to straight chain ABS by successive transfer

(ii) Pure Cultures Isolated from Natural Sources

In order to investigate the fate of organic chemicals in certain specific natural environments, many investigators have used the approach of isolating microorganisms from a particular environment and using those species in degradation work. Samples obtained from natural water systems are diluted with synthetic medium or sterilized natural water and placed on the agar plates.

Isolated colonies are picked up and propagated on an appropriate medium and used in studying degradation of organic chemicals. The procedure differs from the enrichment culture technique (see the following section) since the medium used for initial isolation is formulated in such a way that it permits many of the organisms to proliferate. Similar to other test methods utilizing pure cultures, these studies individually fail to simulate the natural environment or to provide information on degradative capabilities of microorganisms under conditions which permit interaction with other species. However, if large enough numbers of pure cultures are examined, the studies may provide some indication of biodegradability.

Salient features of the degradation studies performed using microorganisms isolated from natural environments are given in Table 9.

(iii) Pure Cultures Obtained from Enrichment

(a) Enrichment with test chemicals

This test, which is also referred to as the Elective Culture Method, is based on the principle that from any natural population the organisms capable of utilizing a test compound as a nutrient source should increase in number during the enrichment period. The term enrichment refers to the opportunity for outgrowth of certain types of organisms over the others.

Table 9. Pure Cultures Isolated from Natural Sources

Reference	Natural Source	Organism Identified As	Test Chemical Studied	Conc. of Test Chemical	Period of Incubation	External Carbon Source
Matsumura *et al.* (1971)	Lake Michigan and its tributaries	approx. 450 isolates (unidentified)	DDT	10^{-5}M	30 days	yeast extract
Strzeiczyk *et al.* (1972)	Eutrophic Lake Jeziorak, Poland	*Arthrobacter* sp. *Corynebacterium* sp. *Nocardia* sp. *Bacillus* sp. unidentified	Aromatic compounds e.g. p-Hydroxy benzoic acid, phthalic acid, salicylic acid, etc.	0.01%	6 days	None
Gonzalez *et al.* (1972)	Solar brine pond, Great Salt Lake, Utah	Bacterium T-52 (unidentified)	Ethylene glycol	10 ml/ℓ	3-5 days	± glucose
Alexander (1973b)	Sea water and sediment material from Connecticut coast	100 marine bacteria	DDT	0.5 µg/ml	7-18 days	yeast extract
Soli (1973)	Not given	5 Bacterial strains (unidentified)	Synthetic oil containing normal paraffins, isoparaffins, cycloparaffins and aromatics	--	10 days	None
Walker & Colwell (1973)	Chesapeake Bay water & sediments	*Cladosporium* sp. *Penicillium* sp. *Alternaria* sp. *Trichoderma* sp. unidentified bacteria and actinomycetes	Motor oil (non-detergent)	10 gm oil powder/ℓ	21-28 days	None
Walker *et al.* (1973)	Chesapeake Bay	*Cladosporium resinae*	aliphatic and aromatic hydrocarbons, organo phosphorus & chlorinated hydrocarbon pesticides	Solids 1% w/v Liquid 10% w/v	30 days	yeast extract

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In this method a source of natural population (e.g., river or lake water, sewage effluent, etc.) is enriched by the addition of low, non-toxic concentrations of the test compound. Transfers are made into fresh medium and enrichment is continued. After several such transfers, the enriched organisms are isolated by streaking on agar medium containing the test chemical, and single colonies are picked. Although the isolation of an organism which uses the test chemical as the sole source of carbon and energy is in itself an indicator of extensive biodegradability of the organic compound (Prochozka and Payne, 1965), most researchers, as an added proof, have further studied the biodegradation of the test compound employing the isolated species in the shake culture test or in a respirometer. Organisms isolated by enrichment culture technique have been utilized extensively by investigators in establishing the pathways of degradation of synthetic organic chemicals. A voluminous literature is available on the use of enrichment culture technique in isolation of an organism for studying the breakdown of chemicals. Some of these studies are summarized in Table 10.

(b) Enrichment for Cometabolic Degradation

Cometabolism is defined as the metabolism of a substrate by a microorganism which cannot use that substrate as a nutrient. This term was first provided by Jensen (1963) and later emphasized by Alexander (1967b) (also see Horvath, 1972a).

Cometabolism may account for the degradation of many synthetic chemicals which do not sustain microbial growth and evidence for the ecological significance of cometabolism is rapidly increasing. The elective culture method as it is normally used completely ignores cometabolism as a factor in the decomposition of organic chemicals. Several investigators have recently attempted to measure the contribution of cometabolic degradation. Organisms can be enriched by application of biodegradable analogues of the pollutant (analogue enrichment) or by adding a co-substrate in addition to the test chemical (co-substrate enrichment) to a natural mixed population of microorganisms.

Focht and Alexander (1971) employed the elective culture technique to obtain microorganisms capable of growing on diphenylmethane, a non-chlorinated analogue of DDT. The ability of the isolated species (a strain of Hydrogenomonas) to grow in a media containing DDT as the sole carbon source or cometabolize it was investigated (Focht and Alexander, 1970, 1971). Horvath and Alexander (1970) obtained twenty isolates representing nine bacterial genera by enrichment culture, and these isolates metabolized substituted benzoates which failed to serve as the sole carbon sources for growth.

Horvath (1973) later investigated the ability of a structurally unrelated substance to serve as an enrichment agent when added in the presence of the test compound. The

Table 10. Degradation Studies Using Pure Culture Isolated from Enrichment

Reference	Test Chemical	Concn. During Enrichment	Source of Mixed Inoculum	Organisms Isolated	Degradation Test	
					Conc. of Test Chemical	Time of Incubation
Payne & Feisal (1963)	Dodecyl sulfate Dodecyl benzene sulfonate	1% solution of 95% sodium dodecyl sulfate or dodecyl benzene sulfonate	Soil near the outfall of sewage disposal plant.	Unidentified, designated C12, and C12B (*Pseudomonas* sp.)	0.015-0.025 M SLS 0.1 M DBS	30 hours
Payne (1963)	"	"	"	bacterium C12B	0.1% (w/v) straight chain saturated alcohol, carbon atom 6-18	72 hours
Prochazka & Payne (1965)	"	"	"	"	various concns. of sodium dodecyl sulfate, dodecanol, Mixture of C_{10}-C_{20} secondary alcohol sulfate	24-48 hours
Bernarde *et al*. (1965) Horvath & Koft (1972)	Alkyl benzene sulfonate	0.1%	—	HK-1 (*Pseudomonas* sp.)	0.1%	24 hours
Forsberg & Lindquist (1967)	Nitrilotriacetic acid[a]	--	River water	Unidentified	as sole carbon source, 1 g/ℓ; as sole nitrogen source, 0.5 g/ℓ	225 hours
Cook (1968)	JNX	10 mg/ℓ	Agar slopes inoculated with sewage work effluent	4 unidentified gram negative rods 2 *Klebsiella* sp.	12 mg/ℓ	16 weeks
Rogers & Kaplan (1970)	LAS	30 mg/ℓ	Activated sludge	20 bacterial isolates belonging to genera *Pseudomonas*, *Achromobacter*, *Paracolobacterium*	Same as in shake culture test of Soap & Detergent Association	
Kipin *et al*. (1970)	LAS	1 gm/ℓ	Soil, sewage, river waters, scrapings from laundry drains, woodland soil	Mixed culture containing various organisms	30 μg/ml	72 hours
Focht & Williams (1970)	p-Toluenesulfonate[a]	1 g/ℓ	Sewage effluent	*Pseudomonas* sp.	0.2-1 g/ℓ	120 hours
Enzinger (1970)	Alpha trinitrotoluene	Subcultured in increasing TNT concn. up to 100 ppm	Mixed liquor suspended solids from the TNT test unit	*Pseudomonas* sp.	100 ppm	5 days
Focht & Joseph (1971)	Nitrilotriacetic acid	0.01 M	Sewage effluent	*Pseudomonas* sp.	0.01 M	10 days for growth; 4 hours for degradation by resting cells
Ahmed & Focht (1973)	Biphenyl p-Chlorobiphenyl	0.1%	Sewage effluent	*Acromobacter* BP *Acromobacter* pCB	0.1%	66 hours
Baggie *et al*. (1972)	Phenylalkanes	—	—	*Pseudomonas acidovorans* Two *Nocardia* strains	few drops (exact concn. not given)	--
Sikka & Saxena (1973)	Endothal	1 g/ℓ	Lake water and hydrosoil	*Arthrobacter*	250-1000 ppm	18 hours
Kaiser & Wong (1974)	Polychlorinated biphenyls (Aroclor 1242)	0.1%	Hamilton Harbor, Ontario	Unidentified	0.1%	2 months

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[a] As sole source of carbon and sulfur

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co-substrate enrichment technique has been utilized in studying degradation of chlorobenzoates. The enrichment medium containing 25 mg/ℓ chlorinated hydrocarbon substance also received 500 mg of glucose/ℓ which served as the co-substrate.

(c) Enrichment for Marine Microorganisms

In an attempt to investigate the breakdown of organic chemicals in the marine environment, researchers have frequently isolated pure cultures of microorganisms from marine environments by enrichment culture technique. Marine mud and water samples collected from coastal areas are generally used as a source of microbial population. The basal medium for isolation is usually sea water supplemented with inorganic salts, e.g., phosphate and nitrate, in which sea water is deficient. The test compound is added to serve as the sole source of carbon and energy. Organisms capable of utilizing the test material are isolated as described before. Although a large number of chemical contaminants have been detected in oceans, most of the breakdown studies have been restricted to oil pollutants, a major marine contaminant.

Bartha (1970) isolated 40 strains of oil-degrading marine microorganisms from oil-polluted as well as non-polluted sea water and marine sediment using a basal medium (sea water or Bushnell-Haas broth supplemented with 3% NaCl) supplemented with individual hydrocarbons or crude oils as the sole added carbon source. The ability of these microorganisms to degrade crude oil (light and heavy), and aliphatic, alicyclic and aromatic hydrocarbons was evaluated. The crude oils as well as other hydrocarbons were filter-sterilized prior to addition to the shake flasks. Atlas and Bartha (1972) selected two bacteria for further study from those isolated by Bartha (1970) on the basis of their rapid growth and wide range of hydrocarbon utilization. The aim was to establish the order in which individual components are degraded, and to quantitate the overall rate and extent of degradation and mineralization by marine isolates. The degradation studies were carried out using a gas train arrangement mounted on a rotary shaker (described in detail in Section III C.2.e, p. 157). In view of the fact that sea water contains very low concentrations of nitrogen and phosphorus, these researcher studied the degradation of crude oil after the addition of olephilic N and P sources. The method is recommended to promote oil biodegradation on the high seas and may not be suitable as a degradation test method.

Cerniglia _et al_, (1971) investigated the degradation of petroleum by microorganisms maintained in stock culture and found that fungi were superior to bacteria. Perry and Cerniglia (1973a, 1973b) subsequently isolated filamentous fungi _Cunninghamella_ elegans and Penicillium zonatum from coastal water by enrichment with crude oil. The basal medium used in these studies was sea water supplemented with a source of N and P. NH_4Cl was reported to be better for growth than $NaNO_3$.

Reisfeld et al. (1972) obtained a mixed population of microorganisms by enrichment culture technique using crude oil as the source of carbon. The medium was supplemented with yeast extract. The enrichment culture was streaked into supplemented oil agar plates to obtain pure cultures of microorganisms. Degradation was studied with mixed as well as pure cultures. Kator (1973) has enriched mixed cultures of petroleum utilizing marine enriched sea water medium (Miget et al., 1969) supplemented with crude oil. An oil-free suspension of enriched cells prepared from the vapor-grown cells (substrate is provided in the vapor phase instead of being added to the growth medium) was used in degradation experiments.

(iv) Pure Cultures Isolated from Naturally Enriched Environment

When a synthetic chemical enters the environment, one or a group of indigenous populations possessing requisite enzymes or which can synthesize necessary inducible enzymes frequently multiply and make use of the introduced substrate. If a particular chemical is continuously dumped into the environment, the species capable of utilizing the compound will increase in population.

Subsequently there is a much greater possibility of isolating an organism capable of degrading a particular synthetic chemical (if the chemical supports microbial growth) from an environment which has previously been exposed to the chemical of interest. In these studies, organisms have been isolated from such continuously exposed sources by plating appropriate samples either on nutrient medium containing a readily available carbon source or a medium in which the test chemical serves as the carbon source. Isolated colonies are picked and pure cultures of microorganisms are used for degradation work. The isolation procedure is different from the enrichment culture technique since no transfers into fresh medium containing the test chemical are necessary. Studies which have made use of this technique are summarized in Table 11.

Table 11. Degradation Studies Utilizing Pure Cultures Isolated from Naturally Enriched Environment

Reference	Source of Microorganisms	Organism Identified As	Test Chemical Studied	Conc. of Test Chemical	Period of Incubation	External Carbon Source
Stahl & Pessen (1953)	Degraded plastic films	*Aspergillus versicolor*. *Pseudomonas aeruginosa*	Plasticizers	3% for esters 2% for alcohols	7 days	yeast extract
Larock & Oppenheimer (1970)	Oil-soaked soil	Unidentified	Mineral oil kerosene mixture	-	5 days	None
Yanagi & Onishi (1971)	Cosmetic products	23 strains of bacteria 25 strains of yeast 17 strains of fungi (some identified)	Ingredients used in formulation of cosmetic products e.g., hydrocarbons, silicones, alcohols, esters, fatty acids	1%	20 days	None
Cooney & Walker (1973)	Contaminated Jet Fuel Systems	*C. resinae*	n-alkanes	-	20-36 days	None

(v) Cell-free Extract Studies

Degradation studies using extracts of microorganisms have generally been carried out for two purposes: 1. To investigate the pathways of degradation; 2. To study the enzymes involved in degradation. Cell-free extracts are suited for this type of investigation because physical and chemical parameters affecting degradation can be controlled easily and the problem of cell permeability has been bypassed.

Heyman and Molof (1968) used cell-free extracts obtained from Pseudomonas C12B, an organism isolated by enrichment on sodium lauryl sulfate (Payne and Feisal, 1963). The extracts were prepared by grinding the cells with alumina in a chilled mortar. Lijmbach and Brinkhus (1973) investigated the mechanism of biodegradation of secondary n-alkyl sulfates and secondary alkanols, using the cell-free extract prepared from the bacteria isolated by enrichment on 2-butanol. The organisms were tentatively identified as a Pseudomonas species. These researchers used an ultrasonic disintegrator for disrupting the cells.

Pfaender and Alexander (1972) studied the microbial degradation of DDT in vitro in the presence of enzyme systems from two species of bacteria. The selected bacteria included a strain of Hydrogenomonas grown on a biodegradable DDT analogue, diphenylmethane, and an Arthrobacter sp. isolated from sewage by enrichment using p-chlorophenylacetic acid (a metabolite formed from DDT by Hydrogenomonas sp.) as the source of carbon.

(vi) Multiple diffusion chamber of study ineraction among pure cultures of microorganisms

An instrument has recently been developed (EcoloGen Model E-40, New Brunswick Scientific Co., New Brunswick, New Jersey) which can be used to study the combined action of several pure cultures of microorganisms in the degradation of organic chemicals. It consists of a central diffusion reservoir surrounded by four peripheral growth chambers, each sealed with a membrane filter. The pore size in the membrane filter allows only the metabolic product of the interacting population to diffuse to other chambers. A metabolite formed as a result of degradation of a chemical compound by a single microbial species will thus become available for attack by different species in another growth chamber.

4. Continuous Culture Technique

In continuous culture, bacterial population density is regulated by automatic addition of the fresh medium to the bacterial culture. The technique allows an investigator to maintain a steady state growth of microorganisms at a desired rate between zero and near maximal. The continuous culture technique has recently been applied to the study of various degradative processes. One advantage in the continuous culture system is that toxic products and metabolic wastes will not accumulate and their effect on the degradation process will not increase with time. The system is more like the natural aquatic environment in this respect where a continual removal or dilution of the toxic products is expected.

Pritchard and Starr (1973) have utilized continuous culture technique to study oil degradation. In this method, the water or nutrient solution is continuously passed underneath the hydrocarbon layer while maintaining a stable two-phase system in which hydrocarbon floats on the surface of the water column. Aliquots of the culture are continually cycled out of the growth vessel, aerated and recycled back (Figure 12). Studies were performed using mixed bacterial population present in Lake Ontario water as well as with pure cultures of bacteria which became predominant in the continuous culture due to their rapid rate of growth. Several researchers have also utilized the continuous culture technique for enrichment of aquatic microorganisms for use in breakdown studies (Jannasch, 1967).

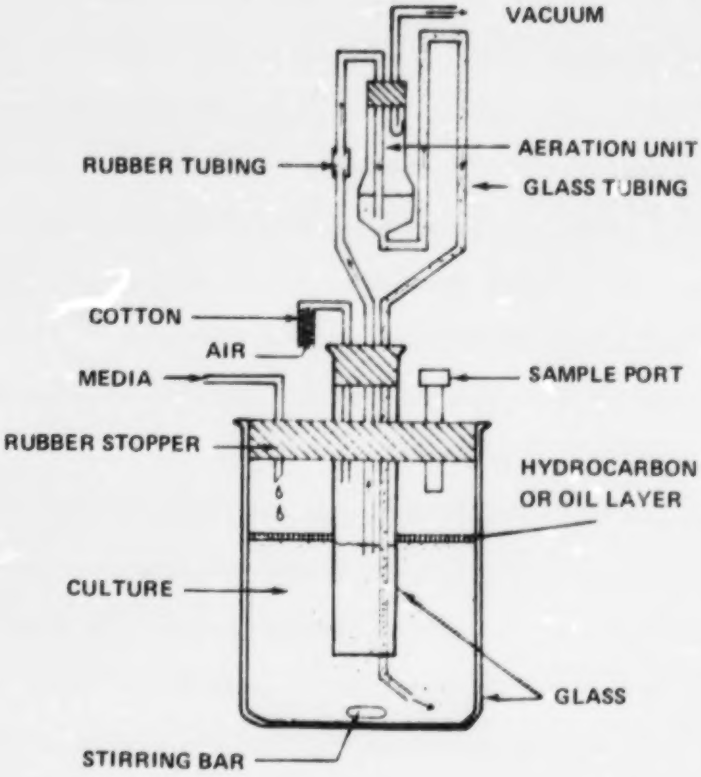
VACUUM
AERATION UNIT
RUBBER TUBING
GLASS TUBING
COTTON
AIR
MEDIA
SAMPLE PORT
RUBBER STOPPER
HYDROCARBON OR OIL LAYER
CULTURE
GLASS
STIRRING BAR

Figure 12. Continuous Culture System (Pritchard and Starr, 1973) Reprinted from Microbial Degradation of Oil Pollutants, Louisiana State Univ.

5. Terrestrial-Aquatic Model Ecosystem

A model ecosystem is a laboratory simulation of a dynamic biological environment characteristic of the natural ecosystem. A large number of contaminants enter the aquatic environment indirectly due to leaching or runoff from the soil. A well-designed terrestrial-aquatic ecosystem could simulate the application of the chemical to a crop in a terrestrial situation and provide for transport from land to a typical lake water situation involving a food web of both herbivorous and carnivorous organisms. By varying the number of the food chain elements, both very complex and simple ecosystems have been developed. The system has been used to examine both the biodegradability and ecological magnification of environmental contaminants.

The laboratory model ecosystem described by Metcalf _et al_., (1971) utilizes a 10 x 12 x 18" aquarium and contains sloping soil/air/water interfaces, plants and food chains of at least 7 elements (Figure 13). The reference water (Freeman, 1953) contains inorganic salts to provide satisfactory mineral nutrition for plants and algae. The model ecosystem contains snails (_Physa_), algae (_Oedogonium cardiacus_), Daphnia and a few milliliters of old aquarium water to provide the plankton. The terrestrial portion of the ecosystem consists of washed white quartz sand molded into a sloping surface into which sorghum is planted. All the food chain organisms are raised in the laboratory.

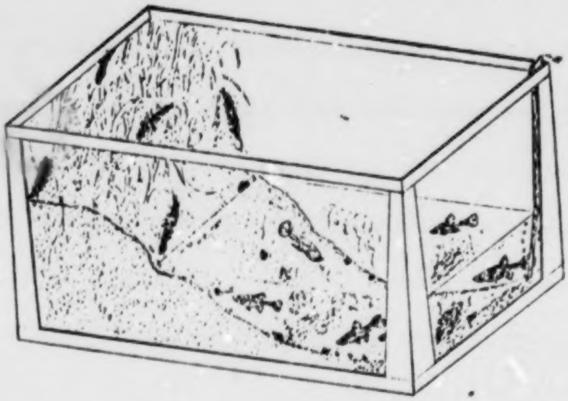

Figure 13. Schematic Drawing of Model Ecosystem for Studying Pesticide Biodegradability and Ecological Magnification (Metcalf, et al., 1971)
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Radiolabeled test compound is added (at approximately the same rate as used in field application) after about 20 days equilibration. Culex quinquefasciatus mosquito larvae are added to the ecosystem after 26 days and Gambusia affinis fish after 30 days. The aquarium is housed in a plant growth chamber with a 12-hour daylight exposure (5000 ft. candles) and the water is aerated continuously. The experiment is terminated after 33 days and the fate of the test chemical in food chain organisms and in the water is determined.

The food-chain pathways for the pesticide in the model ecosystem are:

(1) Sorghum → Estigmene (larva)
(2) Estigmene (excreta) → Oedogonium (algae)
(3) Oedogonium → Physa (snail)
(4) Estigmene (excreta) → Diatoms
(5) Diatoms → Plankton
(6) Plankton → Culex (larva)
(7) Culex → Gambusia (fish)

The authors claim that the model ecosystem techniques provide a considerable possibility for the test contaminant to be degraded by simulated sunlight, air, water, and during passage through the food chain.

Since its first reported use, the model ecosystem of Metcalf et al. (1971) has been used in a great number of other studies to examine biodegradability and ecological magnification of environmental contaminants. The compounds studied have included polychlorinated biphenyls, organochlorine pesticides including DDT and its analogues, phthalate plasticizers, phosphate and carbamate ester insecticides (Metcalf & Lu, 1973; Metcalf, 1974; Kapoor et al., 1972; Hirwe et al., 1972; Kapoor et al., 1970; Metcalf et al., 1973a; Metcalf et al., 1972). The ecosystem has also been used by Metcalf et al. (1973b) to study the interaction among pesticide chemicals.

Sanborn and Yu (1973) have used the model ecosystem described by Metcalf et al. (1971) but modified the aquatic food chain slightly. In addition to the standard components of the Metcalf ecosystem, these researchers also added one crab (Uca minax), two small fingernail clams (Corbicula manilensis) and a water plant Elodea. Yu et al. (1974) used the model ecosystem described by Sanborn and Yu (1973) but in addition included frogs in the food chain. Most of the food chain organisms were obtained from stock cultures in the laboratory except clams, crabs, frogs and Elodea which were obtained from the local pet shop. They investigated the fate of carbofuran in the model ecosystem. Due to the toxic nature of carbofuran, most of the organisms were killed shortly after the introduction of insecticide and, therefore, tanks had to be restocked every 5-7 days. The exposure period for most aquatic organisms was hence reduced.

6. Model Aquatic Ecosystem

Several ecosystems which simulate the aquatic environment alone have been developed to study the environmental fate of those compounds which are either applied or supposedly formed in water. These systems generally contain elements of an aquatic food chain in an aquarium which is filled with natural or reference water (see p. 118) containing inorganic nutrients. The complexity of the ecosystem is generally determined by the food chain pathways simulated in the ecosystem.

One of the simple ecosystems has been described by Sharman (1964) for studying biodegradation of synthetic detergents. The system used a 14 gallon aquarium which contained 44 ℓ of river water. Eleven small fish (guppies, white clouds and cat fish) were introduced and the system was allowed to approach steady state for 7 weeks. In order to stimulate acclimation of the living components, small amounts of the detergent (0.5 mg/ℓ) were initially added. Formal test was started 4 weeks later by the addition of branched and straight chain ABS at the concentration of 1 mg/ℓ.

In the study of metabolism of DDT by natural microbial communities, Pfaender and Alexander (1972) used a water-sediment ecosystem. The system uses a 4-ℓ glass bottle to which two-liter portions of sewage and fresh water containing sediment collected from a rural stream were added. Sterilized air was passed over the surface of the liquid. Autoclaved samples of sewage and fresh water plus sediment were incubated in a similar fashion to show nonbiological change. Sikka and Rice (1973), in their studies on endothall degradation, used 10-gal.

capacity aquaria to which 7-gal. of pond water and 1½" layer of pond hydrosoil was added. An aquarium containing endothall in autoclaved pond water served as control.

The ecosystem devised by Metcalf and Lu (1973) is specially suited for compounds which are volatile. Metcalf (1974) has claimed that the system provides a rapid 3-day evaluation of ecological magnification, biodegradability and degradative pathways as against more complex terrestrial-aquatic model ecosystems (see p. 118). In this system a 3-neck flask (3ℓ capacity) fitted with reflux condenser and two traps to collect volatile products and $^{14}CO_2$ is used. The aquatic fauna and flora (reared in the laboratory) mixed with standard reference water are transferred to the flask. The radiolabelled test compound is added to the ecosystem and after an exposure period of 3 days, analysis is performed.

The aquatic ecosystem has been used by these investigators to study the degradative fate of several industrial organic chemicals such as benzene derivatives, chlorinated biphenyls, and such pesticides as DDT, aldrin, etc.

Isensee and coworkers (Isensee _et_ _al_., 1973; Isensee and Jones, 1974) modified the terrestrial-aquatic model ecosystem of Metcalf _et_ _al_. (1971) to make it suitable as an aquatic ecosystem to study the environmental fate of aquatic contaminants. These researchers eliminated the terrestrial phase from the ecoystem and modified the standard reference water by increasing the NH_4NO_3 and K_2HPO_4 concentration five-fold to obtain satisfactory algal growth. Since the major mode of entry of certain pesticides into water systems is from erosion of pesticides adsorbed on soil, the researchers adsorbed the ^{14}C-labelled compounds on soil and then placed the soil in the aquarium tank and added water. The temperature in these studies was maintained at about 21°C (since 21°C is the optimum temperature for one of the food chain organisms) unlike the Metcalf system, where the temperature was 26°C. In the modified ecosystem, the food chain pathway was as follows: Water → Algae → Snails, and water → diatoms, protozoa, and rotifers → Daphnia → fish.

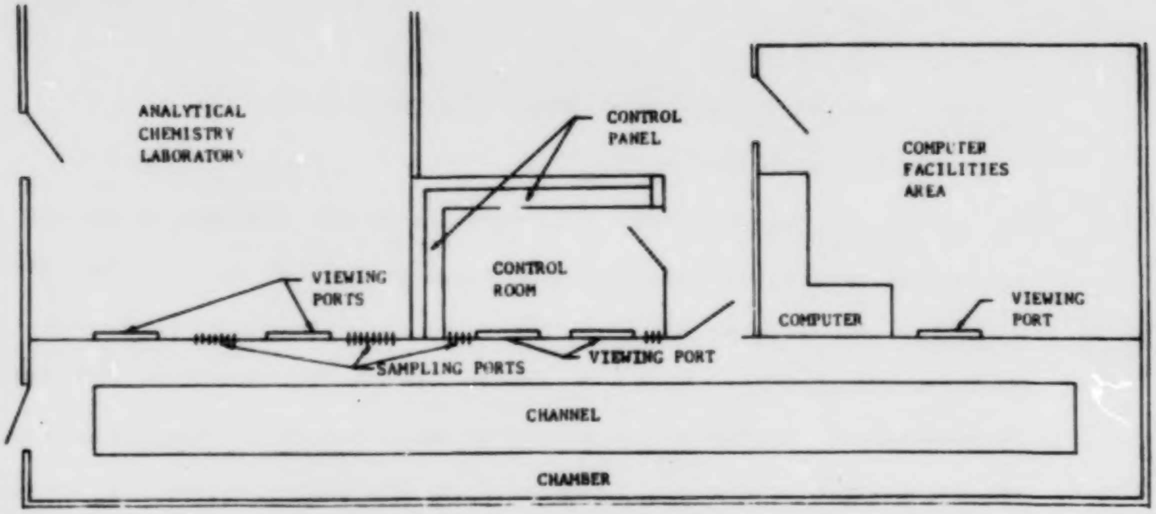
Johnson (1974) has developed a food chain model representing three aquatic trophic levels to study bioaccumulation and biodegradation of xenobiotics. These simulate warm water (>16C) or cold water (<16C) food chains and have the following food chain pathways:

1. Microorganisms → filter feeder (Daphnia) → fish (blue gill) or
2. Detritus (leaves) → scavenger (scud) → fish (rainbow trout), respectively.

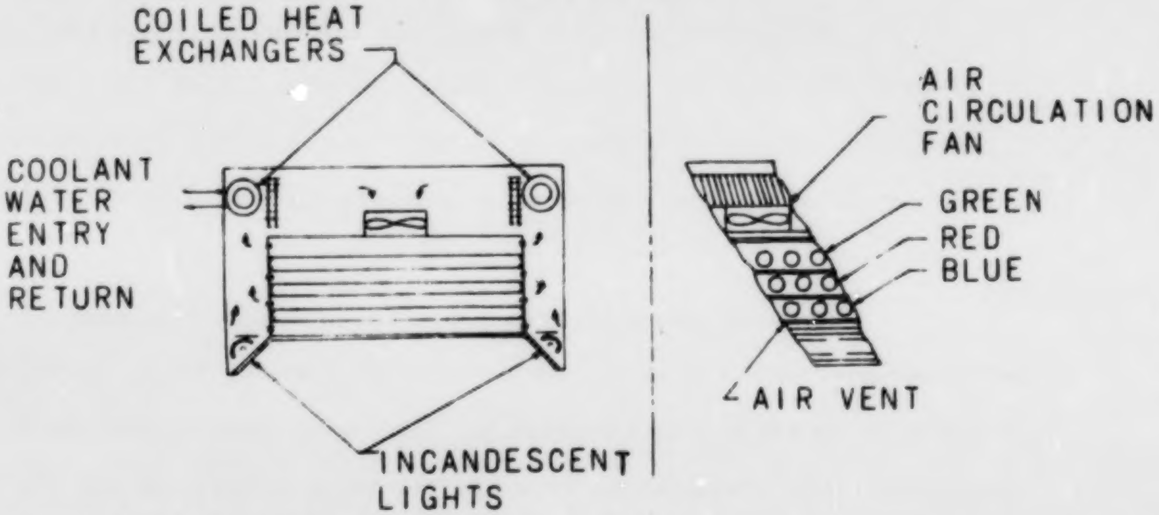
Cochrane et al. (1967) used plastic pools (30" high and 10 ft. in diameter) to study persistence of Silvex. About 8 cubic feet of soil (3 major soil types characteristic of the southeast) was added to the pool within an area 6.5 feet in diameter. The pool was then filled with approximately 80 cubic feed of water. Alligator weed was planted in the pool and, when the growth became established, the herbicide was applied by spraying.

The Southeast Environment Research Laboratory (EPA, Athens) (Sanders & Falco, 1973; Lassiter & Kearns, 1973; Falco & Sanders, 1973) has recently developed an aquatic ecosystem simulator to study ecological processes which involve interactions between chemical and biological systems (Figure 14). The system consists of an environmental chamber (22 meter x 3.66 meter) which houses a water channel (19.5 meter long x 0.46 meter wide x 0.6 meter deep). The level of turbulent mixing in the channel is regulated by rotating paddle wheels. The channel is equipped with a radiant energy system (consisting of red, green and blue fluorescent light, and incandescent light) and an air circulator system. Water is supplied to the channel after passing through a deionizer, still and heat exchanger.

No reports are available at this time concerning the use of aquatic ecosystem simulator for determining environmental persistence of chemicals. E.P.A. has recently announced, however, (EPA, 1974) that studies will soon begin with the simulator to determine net degradation rates under conditions when various modes of degradation are competing under simulated natural conditions. It is anticipated that the studies will enable researchers to predict what happens to a chemical when it is discharged into natural waters.


ANALYTICAL CHEMISTRY LABORATORY
CONTROL PANEL
COMPUTER FACILITIES AREA
CONTROL ROOM
VIEWING PORTS
COMPUTER
VIEWING PORT
SAMPLING PORTS
VIEWING PORT
CHANNEL
CHAMBER

A. Floor plan - schematic of chamber facilities.


COILED HEAT EXCHANGERS
COOLANT WATER ENTRY AND RETURN
INCANDESCENT LIGHTS
AIR CIRCULATION FAN
GREEN
RED
BLUE
AIR VENT

B. Schematic of ecosimulation chamber light source.

Figure 14. Aquatic Ecosystem Simulator
(Sanders and Falco, 1973)
Courtesy of Pergamon Press Ltd.

B. Techniques Simulating Sewage Treatment Conditions

1. Introduction

The techniques discussed previously simulate to varying degrees conditions in nature. However, many researchers have found it very difficult to extrapolate results of those techniques to estimates of removal during waste water treatment because of the very great differences in such parameters as temperature, microbial concentration and types of microorganisms, food source and acclimation. This lack of correlation has led to development of techniques which better simulate actual water treatment conditions.

Water treatment techniques which are currently modelled in the laboratory include activated sludge processes, trickling filter processes and septic tank treatment. A basic understanding of these treatment processes is necessary in order to evaluate the modelling techniques which are used.

Although some physical-chemical waste water treatment techniques are currently being considered (e.g. Lewicke, 1972), biological treatment is by far the most prevalent. Since many chemical substances will pass through such treatment processes before being released to the environment, it is important to understand the degradation which may take place. However, a careful distinction should be made between the "treatability" versus the biodegradability of a chemical substance under treatment conditions. Often a substance is removed during treatment by physical processes such as adsorption on sludge. Although the quality of the water receiving the effluent is improved the overall environmental quality may be reduced since the sludge is often dumped somewhere else (e.g. landfilled or dumped in the ocean) and the chemical may persist in that environment. On the other hand, one method of sludge disposal is incineration and that technique may totally degrade the chemical substance removed by treatment.

Activated sludge systems are one of the most commonly used treatment processes, especially in municipal sewage treatment. The system basically consists of primary removal of suspended solids followed by secondary treatment with aerated activated sludge (see Figure 15). Chemical substances in the effluent stream can be removed (a) by oxidation in the sewer, (b) by absorption on activated sludge followed by oxidation, (c) by adsorption on activated sludge which is then "wasted" as excess sludge (Stennett and Eden, 1971) or the substance may be removed with settled material in the primary clarifier.

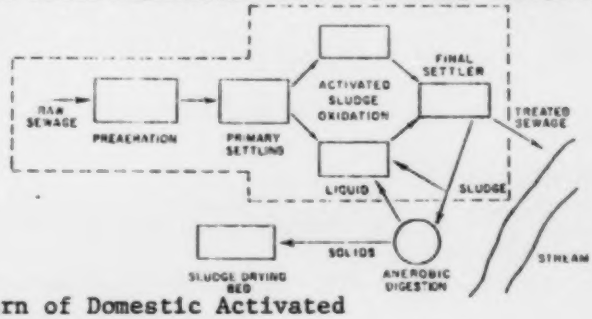

RAW SEWAGE
PREAERATION
PRIMARY SETTLING
ACTIVATED SLUDGE OXIDATION
FINAL SETTLER
TREATED SEWAGE
LIQUID
SLUDGE
SOLIDS
SLUDGE DRYING BED
ANEROBIC DIGESTION
STREAM

Figure 15. Flow Pattern of Domestic Activated Sludge Waste Disposal Plant (Huddleston and Allred, 1964)

Courtesy of J. of the Amer. Oil Chemists' Society.

In terms of biodegradation, oxidation by activated sludge is most important. Activated sludge is a bacterial floc in which are embedded living and dead cells, cell wall fragments, and inert particle material, both organic and inorganic. The concept of activated sludge treatment involves four general steps (1) aeration of the sludge and wastewater for some significant period of time, (2) solid-liquid separation at the end of the aeration period, (3) discharge of the liquid fraction as process effluent, and (4) return of some or all of the separated solids to the aeration stage of the process [concentration of mixed liquor solids (MLSS) is maintained at about 1200 to 3000 mg/l] (Eckenfelder et al., 1972). The time frame for each of these steps can vary considerably. The aerator can be long and baffled and designed for only a single pass from inlet to outlet or it can be a completely mixed system (Swisher, 1970). In some cases the sludge is allowed to absorb contaminants for short periods of time (15-30 minutes), and then separated and aerated for a period of time (commonly 2-6 hours) sufficient for solubilization and metabolism of the sorbed organic matter (contact stabilization). Other designs include extended retention times of 1-2 days which has the advantage of decreasing the amount of sludge disposal (economically attractive for small plants). Another long retention time design is the oxidation ditch which has been used extensively in Europe. The design consists of a circular ditch in which the activated sludge liquor is repeatedly recirculated (Swisher, 1970).

The flocculation ability of the sludge is extremely important to the treatment efficiency, since the separation of the sludge from the effluent and thus the efficiency of removal requires agglomeration and settling of the sludge. Occasionally, when a plant is not properly operated, filamentous microorganisms are formed producing a "bulking sludge" condition which retards settling.

In contrast, trickling filters (percolating filters, bacterial bed) are not dependent upon flocculation. The effluent to be treated is passed over a biological film which has grown on packing material (usually a few centimeters to a number of inches). Although exposure to the film is only a few minutes, soluble material is absorbed on the film and may be degraded over a long period of time (Swisher, 1970).

In both activated sludge and trickling filter processes, the bacteria are the principal agents of biodegradation (Buzzell et al., 1969). Extracellular enzymes solubilize the compounds in order to allow suitable entry into the cell wherein the cell can use the soluble chemical either for energy or to build new cell substances. Higher microbial and invertebrate forms, such as protozoa and worms, feed on the bacteria and maintain a population balance (see Pike and Curds, 1971, for a detailed description of the microbiology of the activated sludge process). With trickling filters, this feeding process appears to be important in prolonging the operation of the filter.

A commonly used sewage treatment process in rural areas is a septic tank system. The system basically consists of a tank which is maintained under anaerobic conditions followed by an underground drainage field or tile field. The anaerobic conditions and short retention time provide little biodegradation and the principal action in the tank is settling of insolubles which are mechanically removed periodically.

2. Activated Sludge Systems

Laboratory activated sludge units are generally of two types (1) continuous and (2) batch or semi-continuous. Because of the large number of procedures reported, only the techniques that have been widely used will be described in detail.

a. Continuous-Flow Systems

The size and dimensions of the activated sludge units have varied depending upon the investigator and the treatment process that is being modelled. Ludzack and Ettinger (1963) have compared the capacity (size of the aerator and settler), the surface to volume ratio, and the dimension of the aerator and settler of a number of units. A recent review of continuous-flow systems is provided by Swisher (1970) in his book on the biodegradation of surfactants. The characteristics of the procedures reviewed by Swisher as well as some newly reported procedures are summarized in Table 12.

Continuous-flow activated sludge systems can be categorized into two general types (1) the serial type aerator which consists of an aerator divided into compartments in series and (2) a completely mixed aerator. The serial type is used in simulation of what Swisher (1970) calls the classical treatment plant; a single pass from inlet to outlet. The configuration used by Eldib (1963) (See Figure 16) is typical of this type of aerator. As can be seen in Table 12, the serial configuration was popular in the late 1950's and early 1960's, but is infrequently used now.

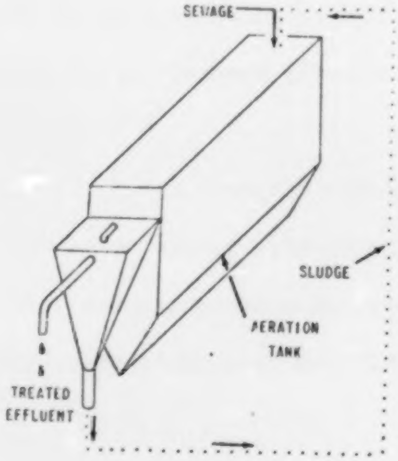


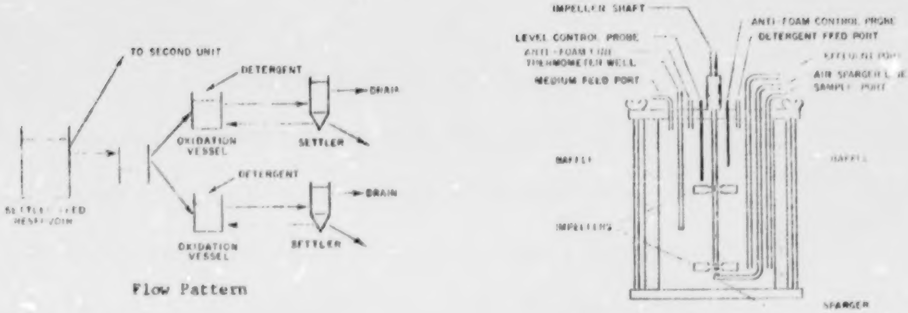
Figure 16. Schematic Diagram of a Serial Type Aerated Chamber Laboratory Model Activated Sludge Unit (Eldib, 1963).

Courtesy of Soap/Cosmetics Chemical Specialties, Mac Nair-Dorland Co., Inc.

Table 12. Characteristics of Bench Scale Continuous Activated Sludge Units

Reference	Capacity Aerator/ settler (liters)	Aerator Dimensions	Settling Dimensions	Dissolved O_2 Level (ppm)	MLSS (ppm)	Feed	Test Chemical	Retention Time	Acclimation	Analytical Method
Truesdale *et al*. (1959)	6.5/0.5	4 sections vertical baffles		2-3	3000	Natural sewage	Surfactants up to 13 ppm	6 - 8 hr.	6 weeks (for TBS or LAS)	Methylene Blue Active Substances (MBAS)
Urban *et al*. (1965)	4.7/	5 compartments in series	conical with rotary scraper	1-2	3000	Synthetic sewage (500 ppm nutrients)	Surfactant 15 ppm	11 hr.	4-12 weeks	MBAS
Husman *et al*. (1963) Jendreyko and Rumchenberg (1963)	200/400 also 2/4	4-50 ℓ compartments in series				Natural sewage (4800 ℓ/day)	Surfactant 20 ppm	1 hr.		MBAS
Degens *et al*. (1955)	15/	completely mixed (also used a sludge aerator)		1-2	2400-3000	Natural sewage (5 ℓ/hr) (BOD 325 ppm)	Surfactant up to 30 ppm	3 hr. (1.5 hr. in settler)	1-2 weeks	MBAS
Huddleston and Allred (1964)	10/	complete mixing (also a preaerator)			2000-3000	350 ppm soy peptone 10% natural sewage	Surfactant 20 ppm	2 - 8 hr.	LAS or TBS 1 week	MBAS
McGauhey and Klein (1959)	1.6/ 10/ 9.6/	6-1.6 ℓ in series			2000-6000	Synthetic and Natural	TBS 5 to 10 ppm 25% removed in synth. 30% removed in natural			MBAS
McKinney and Donovan (1959	1.25/0.25	Completely mixed rectangular shape			1000-2000 (6 ℓ/day)	Natural and synthetic		7.5 hr.		MBAS
Ludzack (1960)	5 ℓ total	Cylindrical shape			3000-6000 (8 ℓ/day)	(200 ppm nutrient broth)				
Renn *et al*. (1964)	3.75/0.75	Rectangular shape				sewage 4 ℓ/day				
Eldib (1963)		Several compartments separated by baffles		2 mg/ℓ	1500		Surfactant 20 ppm	3-6 hr.		MBAS
Pitter and Tutko (1964)	6/0.5				2000-3000	240 ppm bacto-peptone + 44 ppm KH_2PO_4	Surfactant 30 ppm	8 hr.		MBAS
German Government (1962)	3/2.2	Complete mixing		2	3000	Synthetic sewage 250 ppm nutrients 1 ℓ/day	Surfactant 20 ppm		Sludge developed in presence of the surfactant	MBAS
Sweeney and Foote (1964)	0.6/0.1	Cylindrical with turbine agitator (150 rpm)		2	500-2000	Natural sewage 2 ml/min.	Surfactant 3 ppm	6 hr.	8-9 day operation	35S radio-labelled surfactant
Swisher *et al*. (1964) Swisher *et al*. (1967a) Swisher *et al*. (1967b)	0.3/0.075	Cylindrical (50 mm)	Cylindrical (27 mm)		2000-6000	Synthetic sewage (150 ppm peptone) or Natural	LAS, ABS up to 200 ppm	3-7 hr.		MBAS Ultraviolet absorption Ferric iron chelation (NTA)
Bussell *et al*. (1969)	/0.35 gal.	Cylindrical 18" diameter	Cylindrical 15" deep	> 5 mg/ℓ	2500	Synthetic sewage glucose 160 mg/ℓ peptone 160 mg/ℓ urea 28.6 mg/ℓ	Organic chemicals 180 mg/ℓ as C		Acclimated to sewage but not test chemical (use lyophilized sludge) slug loading	TOC, COD Dehydrogenase enzyme activity
Stennett and Eden (1971)	3/		Porous-pot technique		2500	Synthetic sewage	Surfactants 5-20 mg/ℓ	3 hr	3-4 weeks	MBAS
Choi *et al*. (1974)	2.34/ 2.45	Complete mixing		4		Natural sewage (clarified)	PCB's 1.63 ppm	5-10 hrs.	Slug loading	Extraction followed by gas chromat.

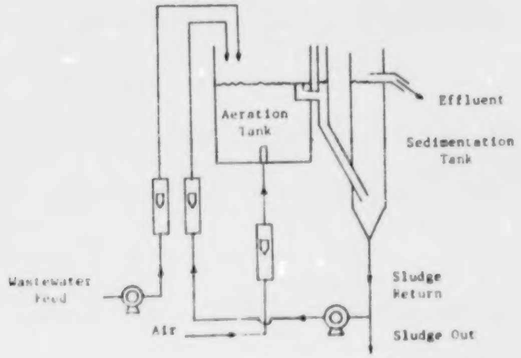
Figure 17 depicts some of the apparatus used in completely mixed systems. The most automated system is the Huddleston-Allred (1964) system. The level is controlled automatically, the foam is repressed automatically, and even the analysis for surfactant is made with an AutoAnalyzer. However, of the completely mixed systems, the best known are the official German test method and the miniature continuous flow units.


TO SECOND UNIT
DETERGENT
DRAIN
OXIDATION VESSEL
SETTLER
DETERGENT
DRAIN
SETTLER FEED RESERVOIR
OXIDATION VESSEL
SETTLER
IMPELLER SHAFT
LEVEL CONTROL PROBE
ANTI-FOAM LINE
THERMOMETER WELL
MEDIUM FEED PORT
ANTI-FOAM CONTROL PROBE
DETERGENT FEED PORT
EFFLUENT PORT
AIR SPARGER LINE
SAMPLE PORT
BAFFLE
BAFFLE
IMPELLERS
SPARGER

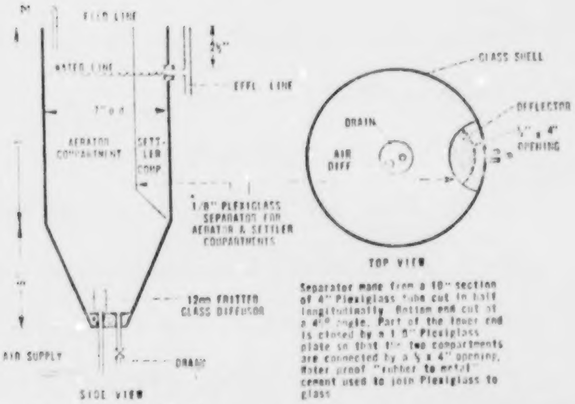
Flow Pattern

Oxidation Vessels

(Huddleston and Allred, 1964)


Aeration Tank
Effluent
Sedimentation Tank
Wastewater Feed
Sludge Return
Air
Sludge Out

(Choi et al, 1974)


FEED LINE
2½"
WATER LINE
EFFL. LINE
7" o.d.
AERATOR COMPARTMENT
SETTLER COMP
1/8" PLEXIGLASS SEPARATOR FOR AERATOR & SETTLER COMPARTMENTS
12mm FRITTED GLASS DIFFUSOR
AIR SUPPLY
DRAIN
SIDE VIEW
GLASS SHELL
DEFLECTOR
½" x 4" OPENING
DRAIN
AIR DIFF
TOP VIEW

7" o.d. Pyrex Shell 3/8" wall drawn to a cone
Corning Glass Works
Spl. App. Div. Reference
No. 0459051 - T140

Separator made from a 10" section of 4" Plexiglass tube cut in half longitudinally. Bottom end cut at a 45° angle. Part of the lower end is closed by a 1/8" Plexiglass plate so that the two compartments are connected by a ½ x 4" opening. Water proof "rubber to metal" cement used to join Plexiglass to glass

Reproduced from best available copy.

(Ludzack, 1960)

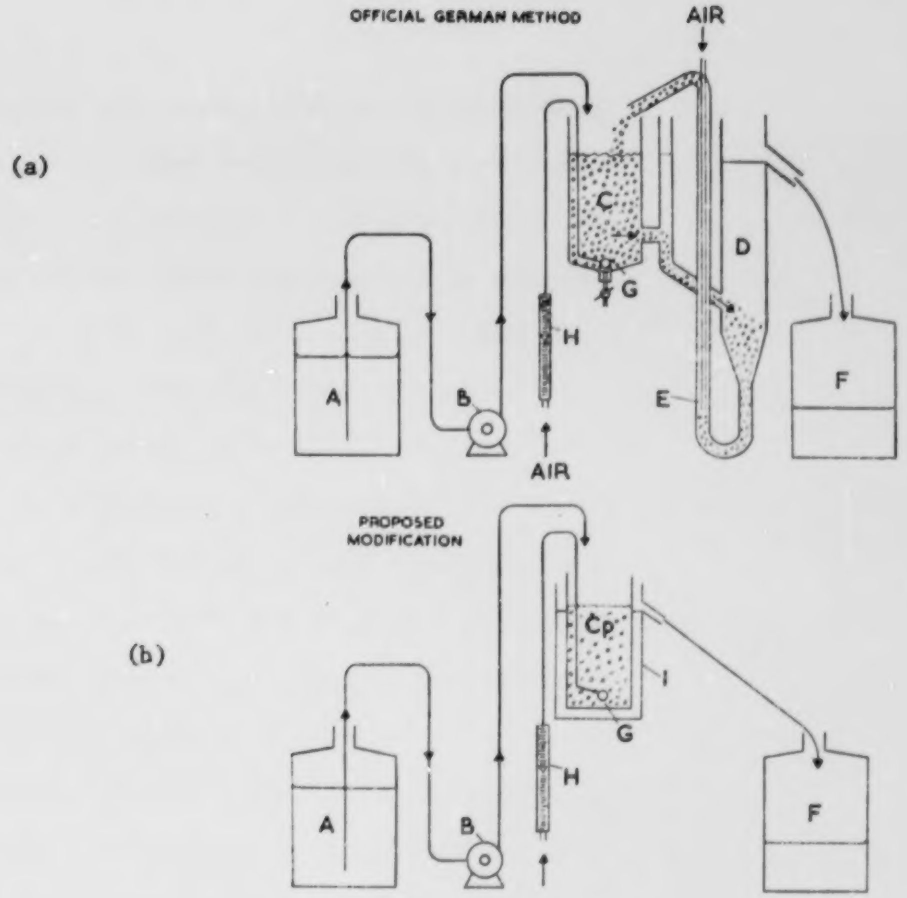
Figure 17. Various Completely Mixed Aerator Model Activated Sludge Unit.
Courtesy of J. of the American Oil Chemists' Society

(i) Official German Test Method

In 1962, the German Government, because of high detergent concentrations in rivers (see Houston, 1963), passed a law requiring that detergents had to be at least 80% biodegradable as demonstrated by the Official German test method. The details of this standard test are described below:

The apparatus used, depicted in Figure 18(a), consists of a complete mixing aerator (3ℓ) and settler (2.2ℓ) with an air pump sludge return. A synthetic sewage containing 250 ppm of nutrients and 20 ppm of surfactants is fed at a rate of 1ℓ/hr. The air flow into the aerator provides agitation and is adjusted to maintain a dissolved oxygen level at above 2 ppm (Swisher, 1970). The sludge is developed as a result of chance inoculation from the air and is allowed to build up to a concentration of about 2500 mg/ℓ. It is presumed that the sludge is acclimated since it is developed in the presence of the test chemical.

The time between development of the sludge and the start of the test period is left to the discretion of the investigator What is required is a resonable steady removal of detergent over a 21 day period, the average removal representing the biodegradability. The effluent is collected in 24 hour composite samples and analyzed for MBAS content. The percent degradation is calculated by comparing the influent content to the effluent content and the 21 daily values are averaged. The minimum time required for the test is about seven weeks



(a)
OFFICIAL GERMAN METHOD
AIR
C
D
G
H
A
B
E
F
AIR
PROPOSED MODIFICATION
(b)
Cp
I
G
H
A
B
F

Figure 18. Apparatus for activated-sludge test: A, storage vessel; B, metering pump; C, aeration vessel; Cp, porous aeration vessel; D, sedimentation vessel; E, air-lift pump; F, effluent collection vessel; G, sintered-glass diffuser; H, air-flow meter; I, outer impermeable vessel. (Stennet and Eden, 1971)

Courtesy of Pergamon Press Ltd.

if everything goes well - about four weeks for sludge generation and three consecutive weeks for the test (Houston, 1963). This procedure has been adopted by the Organization for Economic Cooperation & Development (OECD) and the Council of European Communities (Council Directive, 1973).

Stennet and Eden (1971) have suggested a "porous-pot" technique modification of the German method addressed at overcoming difficulties with recirculation of the sludge. The apparatus is depicted in Figure 18(b). The basic difference is that the sludge is separated from the effluent by retention in a porous polythene vessel (average pore size 50 μm) rather than by flocculation and settling in a clarifier. All other procedures are identical to the official German test with the exception that the porous vessel needs to be cleaned (1:6 diluted hypochlorite solution) once a week. The mixed liquor is transferred to a spare porous vessel to allow continuous operation. This porous pot modification has been used by a number of researchers from the Water Pollution Research Laboratory, Stevenage, England (Eden _et_ _al_., 1972; Stiff and Rootham, 1973; Stiff _et_ _al_., 1973) to study the effect of temperature on the removal of surfactants during sewage treatment.

(ii) Miniature Continuous-Flow Units

Two miniature activated sludge units have been reported: (1) the 600 ml aerator size used by Sweeney and Foote (1964) and (2) the 300 ml aerator size used extensively by Swisher (1964, 1967a, b).

The configuration of the two apparatus is quite different as can be seen by comparison of Figure 19 and 20. However, both systems add the influent to the top of the aeration chamber and provide agitation and circulation by bubbling air from the bottom of the aeration chamber. However, Sweeney and Foote (1964) felt that it was also desirable to provide gentle stirring (150 rpm) in order to maintain agitation without having to keep the air flow rate high.

Sweeney and Foote (1964) elected to use a natural sewage feed with an activated sludge from a commercial activated sludge treatment plant (same source as the sewage). The sewage was a settled sewage obtained from a commercial plant and sterilized at 120°C for 0.5 hr. The activated sludge was obtained immediately before every run. This use of natural sewage and sludge shortens the acclimation time required (two days elapsed before samples are taken). The residence time is 6 hr. and the test can be run for as little as 8 or 9 days if "(a) fresh commercial sludge is used, (b) fairly steady values for surfactant removal are obtained and (c) the results are normalized by using a control" (standard surfactant for normalization) (Sweeney and Foote, 1964). The test surfactant is introduced at 3 ppm so that the total surfactant level (test surfactant and surfactant in the natural sewage) would not be abnormally high (6-11 mg/l typical in U.S.). Because of the background surfactant in the natural sewage, radiotracer techniques (^{35}S) are used for analysis (ether extract of acidified sample).

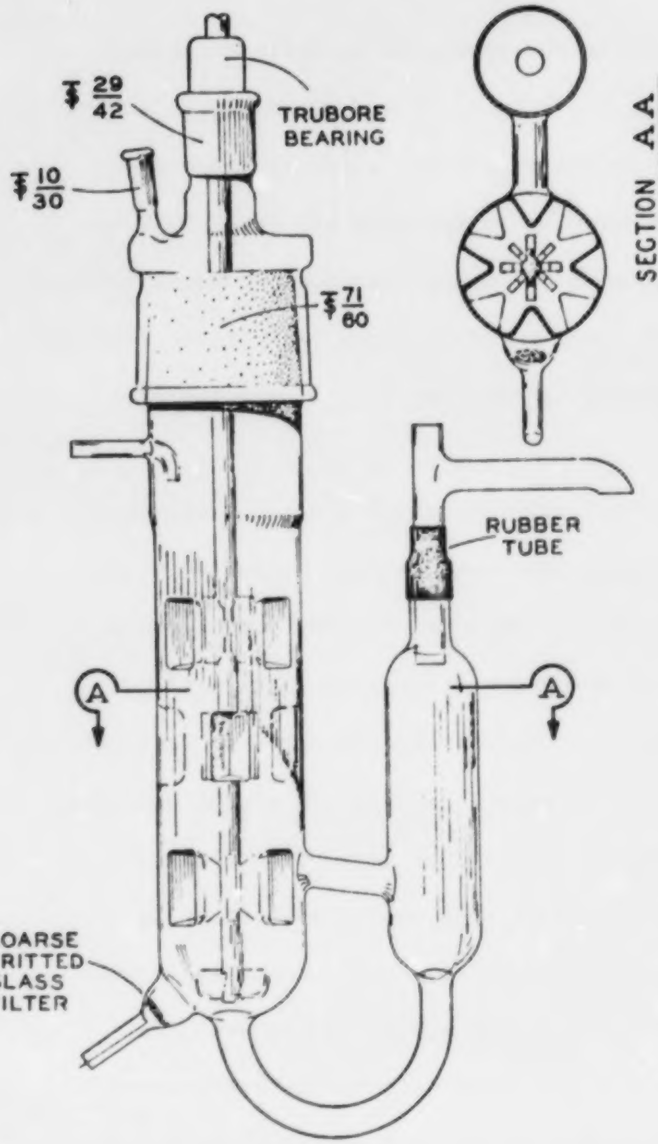

29/42
TRUBORE BEARING
10/30
71/60
SECTION AA
RUBBER TUBE
A
A
COARSE FRITTED GLASS FILTER

Figure 19. Sweeney and Foote (1964)
Continuous Activated Sludge Unit
Reprinted with permission from
Journal Water Pollution Control
Federation, 36, 14-37, Wash., D.C.

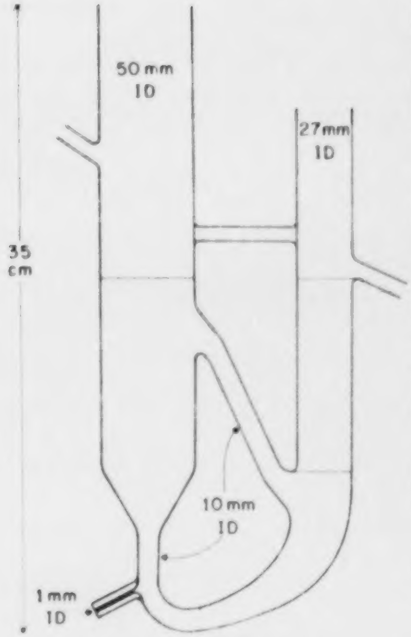

50 mm ID
27mm ID
35 cm
10 mm ID
1 mm ID

Figure 20. Swisher (1964, 1967a, b)
Miniature Complete Mixing
Continuous Activated
Sludge Unit
Reprinted from Surfactant
Biodegradation, p. 169, by
courtesy of Marcel Dekke
Inc.

The miniature activated sludge unit used by Swisher (1964, 1967a, b) is about half the size of the Sweeney and Foote (1964) unit. Swisher has studied the biodegradation of linear alkylate sulfonates (LAS) and nitrilotriacetic acid (NTA) with these units and Gledhill (1974) used the apparatus to study the biodegradation of 3,4,4'-trichlorocarbaniiide. The activated sludge cultures, originally from a municipal treatment plant seed, are developed on and acclimated to a synthetic sewage and the test chemical for several months. Swisher (1970) also notes that natural sewage can be used with this unit. The analytical method used is dependent upon the chemical studied and study objectives. Swisher used a combination of the MBAS method and ultraviolet absorption to study the breakdown of the LAS benzene ring (1967b) and a chelometric technique for NTA (1967a). Gledhill (1974) determined the biodegradation by $^{14}CO_2$ evolution.

b. Semicontinuous and Batch Systems

(i) Batch Systems

Continuous activated sludge systems require a great deal of effort to set up and keep running and therefore many investigators have elected to use batch and semi-continuous systems. Swisher (1970) has noted that the batch unit is much more economical, although somewhat more remote from simulation of full scale practice.

The simplest test to run is the one batch activated sludge die-away. This consists of aerating a sample containing activated sludge, sewage (natural or synthetic), and the test compound and analyzing for the loss of the chemical over a few days or weeks. Actually, with exception of the feed (activated sludge and sewage), this technique is little different than a river die-away test (see Sec. III A.2, p 72). Swisher (1970) has reviewed the conditions of a number of batch sludge die-away systems and these are presented in Table 13. One interesting variation of this technique is reported by Gledhill (1974). He studied the biodegradation of radiolabelled 3,4,4'-trichlorocarbanilide (200 μg/l) using a shake flask apparatus, raw sewage, and activated sludge (MLSS - 1000 mg/l) from a semicontinuous activated sludge unit (only natural sewage feed). The shake flask was a closed system which allowed the monitoring of $^{14}CO_2$ evolution by KOH absorption.

(ii) Semicontinuous Systems

By operating a batch system in series (running batch after batch), a semicontinuous system evolves. This is often termed a fill-and-draw process. The process basically consists of (1) aeration (2) settling of sludge (3) drawing off the supernatant liquor (treated effluent) (4) filling with fresh feed and (5) aerating the new cycle. Analysis is normally done at the end of the cycle. Thus, the fundamental differences between a batch and a semicontinuous process is that the sludge has an opportunity to acclimatize to the test compound since it is retained from cycle to cycle. Interestingly enough,

Table 13. Batch Sludge Die-Away
(Swisher, 1970)

Reference	Test Compound	Aeration	Feed	% Removed
Sierp and Thiele, 1954	Alkyl sulfate	Bubbled air - 3 hrs.	Activated sludge & sewage	40-60%
		Bubbled air - 3 hrs.	No sludge	4%
		No aeration	Activated sludge & sewage	0%
	Alkylaryl sufonate	Bubbled air - 3 hrs.	Activated sludge & sewage	80-90%
		" "	No sludge	70-80%
		No aeration	Activated sludge & sewage	9%
House and Fries, 1956	TBS	Bubbled air - 8 hrs.	Activated sludge & sewage	25-85%, depending on sludge adaptation
Vaicum and Ilisescu 1967 (Romanian ISCH Test)	Alkyl sulfate & ABS	Bubbled air - 3 days	Unacclimated sludge (MLSS-1000-1500 ppm) Official German synthetic sewage	"Closely paralleled results from 6 tests (river water, 3 continuous activated sludge, BOD, and Warburg" (Swisher, 1970)

the semicontinuous process had its beginnings in the acclimation of sludge seed for Wárburg respirometry (Swisher, 1970).

By far the most commonly used semicontinuous process is the standard procedure developed for ABS-type surfactants by the Soap and Detergents Association's Subcommittee on Biodegradation (SDA, 1965). The full standard procedure consists of a two-step approach (1) a simple shake flask screening or presumptive step (two 72 hr. adaptive transfers) (see p. 85), and (2) a semicontinuous activated sludge step. If the surfactant is only 80-90% removed in the screening step the semicontinuous confirming test must be run. If the surfactant is >90% removed, it is considered to be adequately biodegradable; if <80%, it is considered not adequately biodegradable for a surfactant.

The semicontinuous step consists of a 24 hr. cycle (23 hr. for aeration; 1 hr. for setting, drawing off the effluent, and filling with fresh feed). The aeration chamber consists of a cylinder with a cone shaped bottom, the dimensions of which are illustrated in Figure 21. The activated sludge, initially obtained from a sewage treatment plant, is maintained at a mixed liquor suspended solids (MLSS) level of 2500 ± 500 mg/ℓ by discarding solids as necessary. The surfactant is added at a concentration of 20 mg/ℓ and if the sludge is not acclimated to the surfactant, the final concentration is built up to in increments over four days. The minimum operation time

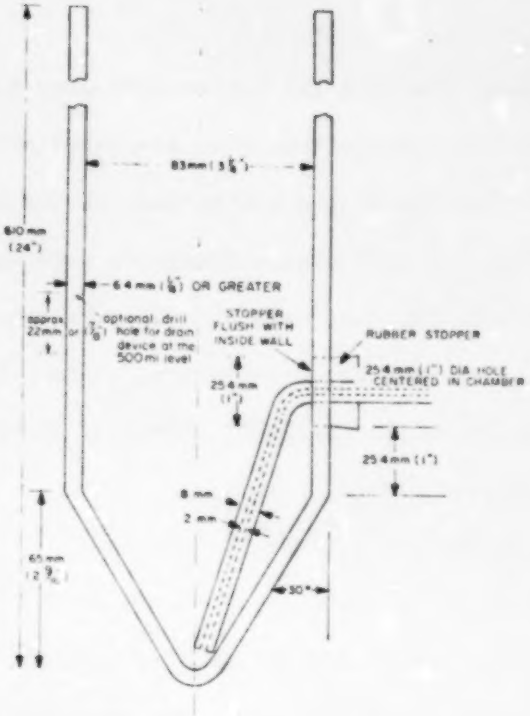

83mm (3 1/4")
610 mm (24")
6.4 mm (1/4") OR GREATER
approx 22mm or (7/8") optional drill hole for drain device at the 500ml level
STOPPER FLUSH WITH INSIDE WALL
RUBBER STOPPER
25.4 mm (1") DIA HOLE CENTERED IN CHAMBER
25.4 mm (1")
25.4 mm (1")
8 mm
2 mm
65 mm (2 9/16)
30°

Figure 21. Soap and Detergents Association's Semicontinuous Activated Sludge Aeration Chamber (SDA, 1965). Courtesy of J. of the Amer. Oil Chemists' Society.

is 15 days - 5 for surfactant build-up, 3 for equilibration to 20 mg/ℓ, and 7 for level operation. A control standard, pure C_{12}LAS, is run in parallel and results are only valid when the standard gives a removal over 97.5%.

The SDA Subcommittee on Biodegradability, with the assistance of twelve laboratories, conducted a statistical evaluation of the semicontinuous procedure with seven ABS-type surfactants. Analysis was determined by the methylene blue procedure. Reproducibility for LAS surfactants was good, but the more highly branched ABS surfactants gave much more varying results. The SDA Subcommittee also evaluated other nonionic surfactants, but because of the ambiguities of the analytical methods no clear-cut performance standards were established (SDA, 1969). Swisher has also used the SDA semicontinuous procedure to study the ultimate biodegradability of LAS (Swisher, 1967b), quaternary ABS compounds (Swisher, 1969) and the biodegradability of NTA (Swisher *et al*., 1967).

3. Trickling Filter Systems

Trickling filter systems have been used extensively by many researchers (e.g. the British Water Pollution Research Laboratory (WPRL) at Stevenage) to study the degradability of surfactants. The start-up period is usually quite lengthy since the biological film which has to be developed on the packing material often requires weeks or months to reach a steady state. On the other hand, difficulties with scale-up are much less since the most important dimension is the depth of the bed (full scale - 6 ft. or 180 cm.) (Swisher, 1970). Truesdale and coworkers have shown that trickling filters agree well with sewage treatment works (Truesdale *et al*., 1959) and have even used the results as a standard of reference for comparing other laboratory techniques (Truesdale *et al*., 1969). Swisher (1970) has reviewed the conditions used with trickling filters and these are tabulated in Table 14. The rotating tube noted in Table 14 simply consists of an empty hollow tube mounted slightly off horizontal and rotated slowly along its axis. A more elaborate description of the WPRL trickling filter and the recirculating filter reported by Jenkins *et al*., (1967), follows.

Table 14. Trickling Filter Conditions
(Swisher, 1970)

Reference	Dimensions of the Filter	Dimensions of the packing	Feed Rate vol. feed/ vol. filter/ day	Feed	Development of Biological Film	Acclimation to Surfactant	Surfactant Concentration
Trickling Filters							
Truesdale et al. (1959) (WPRL)	15 x 180 cm	2.5 cm	average 0.6 diurnal variations	Detergent free natural sewage	14 weeks	4-8 weeks	13 ppm
Husman et al. (1963)	15.5 x 55 cm	4 cm	1.0	Natural sewage or peptone, glucose, salts synthetic sewage			10-200 ppm
Schonborn (1962a)	10 x 110 cm		1.0	Develop film on natural. Test on synthetic	4 weeks		15 ppm
Rotating Tube							
Gloyna et al. (1952)	6.5 x 60 cm						
Weaver (1962)			3 l/day	Synthetic sewage			10 ppm
Renn (1965)			5 gal/day	Synthetic sewage			10 ppm
Recycle Trickling Filter							
Burnop & Bunker (1960)	5 x 90 cm (glass)		unspecif. vol. recycled 7 times per hour	Synthetic (malto-peptone)			10-50 ppm
Edeline & Lambert (1965)	14 x 124 cm	1-3 cm stone (up to 110 cm)	½ - 1 l/hr.	Official German	previously developed	None	20 ppm
Jenkins et al. (1967)	3.75 x 75 cm	5 x 8 mm washed gravel	recycled 7-18 times/ day	BOD water salts 10 ppm NH_3-N			3-20 ppm sole carbon source
Alexandre (1967)	10 x 200 cm	1 cm pozzolane granules (up to 150 cm)				24 hours	

a. British WPRL Pilot-Scale Trickling Filters

The trickling filter extensively used by the British Water Pollution Research Laboratory consists of a cylinder, 15 cm in diameter and 180 cm deep, filled with 2.5 cm clinker (Truesdale, et al., 1959). A detergent-free sewage (∿8 gallons/day) (prepared from excreta and other normal constituents of sewage) is applied to the filters (average 0.6 m^3/m^3 of filter per day) in such a manner as to simulate the diurnal variations in flow at a sewage works ("square wave dosing"). The common pattern is 1.5 times the average flow during the day and 0.5 times the average flow at night (1.5/0.5). This "square wave" dosing was used because uniform loading gave unusually high biodegradability values in the laboratory in comparison to actual practice. However, Klein and McGaughey (1965) using a much less drastic "square wave" loading (1.33/0.67) found no difference between uniform and "square wave" loading after sufficient acclimatization was allowed.

The test is usually continued for several weeks until steady state conditions are reached. In some cases considerable acclimation time must be allowed - usually 5 to 8 weeks, but sometimes 12-13 weeks - and about 14 weeks is required to develop a mature film (Swisher, 1970). The effluent is analyzed for surfactant [usually MBAS although Klein and McGaughey (1965) have used ^{35}S radiolabelled material] and the percent removed is calculated. Truesdale et al (1969) have suggested that "results obtained in this test agree well with those obtained at an efficient sewage treatment works."

b. Recirculation Filter Test

Even small trickling filters require substantial amounts of feed and test chemical during the many weeks or months necessary for attaining steady state and acclimation. To overcome this difficulty many researchers have switched from once through operation to recirculating the effluent in a batchwise procedure. The procedure used by Jenkins and coworkers (1967) is somewhat typical of this approac

The apparatus used by Jenkins et al. (1967) is depicted in Figure 22. It consists of a black PVC tube (length - 75 cm, diameter - 3.75 cm), containing a column of 5-8 mm washed gravel. The feed is a solution of the test chemical (10-20 mg/l surfactant) made up initially in a 1% dilution of sewage effluent or soil-extract in BOD water and 10 ppm NH_3 nitrogen (needed for smooth biological oxidation of detergents). The solution is circulated through the column at a rate varying from 7 to 18 cycles/day and when the test chemical concentration reaches a low level, it is replenished. After several replenishments, the gravel was sufficiently inoculated with microorganisms acclimated to the test chemical that reasonably consistent results

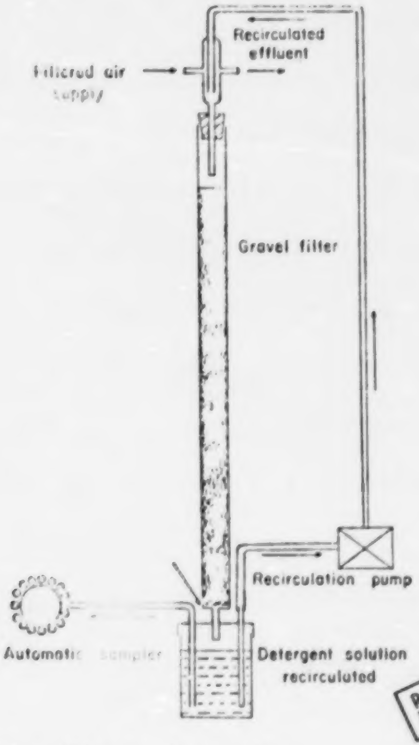

Recirculated effluent
Filtered air supply
Gravel filter
Recirculation pump
Automatic sampler
Detergent solution recirculated

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Figure 22. Recirculation Filter Apparatus
(Jenkins et al. 1967)
Courtesy of Pergamon Press Ltd.

could be obtained. It is at this point that the inoculate (1% sewage or soil) is omitted and the test chemical becomes the sole source of carbon during the test cycles.

4. Anaerobic Systems

Two anaerobic systems which are modelled in the laboratory and are important to sewage treatment are (1) septic tanks and (2) anaerobic digesters. Septic tanks are used in most rural areas and in many suburban areas, Chemical substances passing through these systems potentially could affect the quality of ground and surface water. Anaerobic digesters are used to reduce the volume of the sludge from an activated sludge treatment plant before it is dried.

The simplest procedure is the anaerobic die-away system. Briefly, this consists of batches of feed and test chemical kept in an anaerobic jar for periods of up to a month of two. The conditions used are outlined in Table 15. When anaerobic digester conditions are simulated, high concentrations of activated sludge or digester sludge are used. Hill and McCarty (1967) have used this procedure with digester sludge to study the anaerobic degradation of organochlorine pesticides, although they note that this type of degradation would probably occur only slowly in less favorable natural conditions.

Swisher (1970) has briefly reviewed semicontinuous operation of laboratory simulated septic tanks and anaerobic digesters with surfactants. These semicontinuous conditions are much closer to actual field conditions. Only the two simulated septic tanks and drainage fields used by Straus (1963) and Lashen _et al_. (1967) will be described.

Table 15. Anaerobic Die-Away Procedures
(Swisher, 1970)

References	Apparatus	Food	Surfactant	Length of Test
Wayman & Robertson (1963)	Brewer anaerobic jar	Primary sewage effluent	10-25 ppm	1-2 months
Vath (1964)	Amber bottles	" "	20-100 ppm	2 weeks
Klein (1965)	Graduated cylinder	2 ℓ- " "	25 ppm	40 days
Manganelli _et_ _al_. (1960) Meinck & Bringmann (1961) Pitter (1964b, c)	Container with provisions for gas evolution	Activated sludge & digester sludge (30,000 ppm susp. solids)	100-750 ppm	40 days

The septic tank used by Straus (1963) consisted of two cylindrical compartments (first - 2 ℓ, second 1 ℓ). This was followed by a drain field which consisted of a "series of columns packed with soil to the inlet, then gravel past the inlet, topped off with more soil." The septic tank was seeded with sludge from a full-scale septic tank and feed sterilized raw whole sewage. The surfactant, an alkylbenzene sulfonate tagged with ^{35}S, was added at a concentration of 10 ppm. The feed was added 4 minutes each hour for 16 consecutive hours each day. The average residence time was 5 days.

The Lashen _et_ _al_. (1967) model septic tank-percolation field consisted of a 1 gallon jar septic tank and a 3 in. diameter column of sand (0.5% peat moss added) 2 ft. in height above a water table. The average retention time was 67 hr. and the system was fed 3 times a day at a rate of 2.5 gallons per day per square foot of surface area in the percolation field. The septic tank was initially seeded with mixed liquid from an activated sewage plant and fed a synthetic sewage (official German recipe).

5. Field Tests

The ultimate test of a chemical's biodegradability under water treatment conditions can only be provided by a field trial. However, the expense is so great that field trials are rarely attempted except in the case of detergents. Swisher (1970) has identified at least 30 field tests undertaken with surfactants. These will not be reviewed here. However, since one of the best evaluations of a laboratory technique is provided by a comparison of results with a field test, some of the procedures and difficulties involved in field testing will be discussed.

Swisher (1970) has identified two major parameters which contribute to the uncertainties of field testing surfactants: (1) the inherent variability of the operating parameters of the treatment plant, and (2) analytical interference by surfactants already present in the incoming sewage. The first parameter may result in widely varying treatment efficiencies. Some control over these variations may be gained by comparing the removal of surfactant to the removal of more natural components of sewage (e.g. BOD). The analytical difficulty results from the fact that the commonly used methylene blue procedure (MBAS) is not a specific method (see p. 154). Other analytical methods are available (e.g. IR, desulfonation-GC, radiotracer techniques) but have been infrequently used because of the speed and sensitivity of the MBAS procedure. Two techniques have been used in order to adjust for the deficiencies of the MBAS procedure (1) substitution technique and (2) spiking technique (Swisher, 1970).

In the substitution technique, all the detergents in a service area are replaced by the test detergent. Thus, in theory the MBAS measured should only be the test detergent. However, this replacement is usually quite difficult to accomplish and it may take years before all the detergent supplies are replaced and all the absorbed or trapped previous detergent is removed from the sewer lines.

The spiking technique is much simpler. It requires an addition of the test surfactant just before treatment operation with analysis before addition, after addition, and after treatment. With this technique, blank runs must be run to determine the removal of the already present surfactant and this may be a large source of error.

C. Analytical Procedures

A number of direct (e.g., estimation of parent compound or its metabolite) and indirect (e.g., measurement of growth or oxygen consumption) analytical methods have been used to study biodegradation in the aqueous environment. Although any analytical method can provide data relevant to biodegradability, there are important differences in the utility of the results. For example, some methods reveal nothing about the nature of the intermediate metabolites and, therefore, have somewhat limited application. The analytical procedures involved in the assessment of biodegradability in the aquatic environment are somewhat similar to those used in the soil studies. The major difference is that the extraction and clean-up procedures are relatively less complex in the natural water samples. Biodegradation studies utilizing pure-cultures of microorganisms and cell-free extracts have generally used the same standard methods regardless of the environment the organism was isolated from.

i. Extraction and Clean-up: Aqueous medium is generally much less complex than soil and therefore extraction and clean-up is not always necessary. The requirement for extraction is more dependent on the type of analytical method which the researcher is planning to use for analysis. Sometimes extraction is essential because of certain unusual binding characteristics of a particular compound. The system used for extraction of a chemical from water is largely dependent on the characteristics of the chemical and less dependent on the water sample. The most frequently used technique is partition of the chemical from the water into a water-immiscible organic solvent. For example, hexane has been used for extraction of chlorinated hydrocarbons (Leigh, 1969), chloroform for polyethoxylated alkylphenols (Osburn and Benedict, 1966); 15% ethyl ether in hexane for organochlorine compounds; 20% benzene in hexane for organophosorus compounds; chloroform for carbamate compounds (Eichelberger and Lichtenberg, 1971); a mixture of benzene and ethyl ether (2:1, v/v) for crude oils (Kator, 1973). Desorption of ABS and several other surfactants from microorganisms has generally been accomplished by extraction with hot water (Hartman, 1963; Kelly et al., 1965; Tomiyama et al., 1968), ethanol (Assoc. Amer. Soap and Glycerine Producers, 1961; Maurer et al., 1965) or methanol (Roberts and Lawson, 1958; Fischer, 1962; Bruce et al., 1966). Allred et al. (1964) has suggested boiling of samples with HCl in order to liberate surfactant from bacterial cells. Several researchers have used alkaline conditions - e.g., alkaline aqueous acetone (Gould, 1962), methanolic sodium hydroxide (Huber, 1962, 1968) for desorbing anionics. In certain cases ionic strengths of the test solution is increased by addition of NaCl prior to extraction with organic solvent (Osburn and Benedict, 1966).

2. Analytical technique:

a. Chromatographic Methods: Chromatographic techniques have been used to isolate and sometimes, by co-chromatography, to identify degradation products and the starting test compound. Paper, TLC or column chromatography is generally used. The parent compound and/or the metabolites are extracted from the biodegradation test medium, the extracts are concentrated by evaporation of the solvent, and the residue is chromatographed. Gas chromatography can be successfully used, but its utility is limited to chemicals which can be volatilized and are thermally stable. Extraction and clean-up of the sample is generally required before GC analysis can be performed. In several cases, however, aqueous samples have been directly injected into the GC for analysis (Baird et al., 1974; Baker, 1966). Anionic surfactants are salts and, therefore, are not volatile enough for GC analysis. Several researchers have developed procedures for desulfonation of ABS giving rise to alkylbenzene which can be used with GC analysis (Setzkorn and Carel, 1963; Swisher, 1963). The desulfonation-GC technique was found to be particularly suitable for measuring relative rates of disappearance of the individual components in a complex ABS mixture (Huddleston and Allred, 1963; Swisher, 1963; Allred et al., 1964).

b. Radiotracer Technique: Radioactive isotopes have been extensively used in studies on metabolism and degradation of environmental contaminants. The major advantage of this technique is that metabolites that are formed are also tagged and, therefore, a mass balance of the parent compound and breakdown products is possible. A large number of recognized environmental contaminants are available from commercial sources in radiolabelled form. In certain cases researchers have synthesized the desired chemicals in their laboratory (Metcalf & Lu, 1973). In a majority of degradation studies, compounds tagged with ^{14}C have been used (Metcalf, 1973; Focht and Williams, 1970; Gledhill, 1974; Vath, 1964; Lashen *et al.*, 1966). ^{3}H-ring-labelled compounds synthesized by a relatively simple technique (Hilton and O'Brien, 1964) have also been used in several studies (Kapoor *et al.*, 1970, 1972). Isotope effects are possible with this technique, but would only be encountered if breakage of the ^{3}H-C bond is the rate determining step. Labelling the sulfonate group with ^{35}S has been frequently used in the case of detergents (Robeck *et al.*, 1963; Straus, 1963; Sweeney and Foote, 1964). Biodegradation is measured by determining the amount of inorganic sulfate formed. The major drawback of using sulfonate-labelled material is that no information can be derived about the fate of the ring.

c. *Colorimetric Methods*: The methylene blue method for following the biodegradation of anionic surfactants has been widely used because of its simplicity and accuracy, and because the loss of methylene blue activity closely parallels the loss of foaming ability, one of the major water quality problems from detergents. Also, the fact that about 75% of the U.S. surfactant production consists of anionic materials (SDA, 1969) has contributed to the extensive use of the technique.

Methylene blue (Figure 23) is a cationic dye which in the form

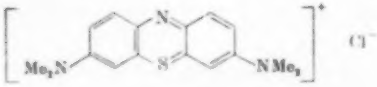

Figure 23. Methylene Blue Dye

of an inorganic salt (e.g. Cl^- or $SO_4^=$) is very water soluble. However, when combined with a surfactant anion it becomes less water soluble and can be extracted by organic solvents. The intense color of the dye allows for detection of low concentration of anionic surfactants (e.g., 10 μg of ABS - 0.1 ppm in a 100 ml sample - can be readily detected).

The procedure, as outlined in Standard Methods (APHA, 1971), consists of additions of the dye solution to the sample followed by chloroform extraction of the surfactant-dye salt. The concentration is determined by measurement of the absorbance of the chloroform solution at 652 mμ (for LAS). The precision and accuracy of the method is acceptable. Standard Methods reports a relative standard deviation of 9.1% and relative error of 1.4% in 110 laboratories with a river water sample which contained 2.94 mg/l of LAS.

The major drawbacks to the procedure are (1) the method only determines the test surfactant and not the metabolites and, therefore, only provides an assessment of primary biodegradation, and (2) the method is not specific for anionic surfactants and can be interfered with by organic sulfonates, carboxylates, phosphates, phenols, inorganic cyanates, chlorides, nitrates, and thiocyanates (complex or form ion pairs with methylene blue - gives high results) and by organic materials especially amines (compete for the methylene blue - low results) (APHA, 1971). Interferences from chloride ions (forces some methylene blue into the chloroform) precludes application of the method in seawater without precautionary measures.

The partition of the surfactant - methylene-blue salt into the chloroform phase is dependent upon the hydrophobic group in the surfactant. Above octylbenzenesulfonates the partition into the chloroform is close to stoichiometric and therefore the method is extremely sensitive for the parent alkylbenzenesulfonates. However, as the side chain is metabolized during biodegradation (Swisher, 1970), the hydrophobic character is reduced and this is why the method is only good for the parent surfactant.

The methylene-blue dye is not specific for anionic surfactant and thus the results are often reported as methylene-blue activated substances (MBAS). In biodegradation tests, this problem is minimized to the greatest extent possible by running a blank that does not contain surfactant.

d. U.V. and I.R. Spectrometry: Measurement of the infrared absorption spectrum can provide information regarding structure, but its relative insensitivity limits any quantitative application. However, an I.R. method based on the measurement of the absorption of the sulfonate group has been used for determination of ABS (SDA, 1965; Ogden *et al*., 1961; Frazee and Crisler, 1964). I.R. spectrometry has also been used in biodegradation studies of nonionics (Frazee *et al*., 1964; Osburn and Benedict, 1966).

The U.V. method of analysis for following the cleavage of the benzene ring has been frequently used in degradation studies in the aquatic environment. The disappearance of U.V. absorption during degradation indicates rupture of the benzene ring system. The U.V. analysis can usually be made directly without concentration or extraction steps. However, in several cases the technique cannot be used if some component of the medium or an impurity absorbs strongly in the region of interest. The concentration of the test material and medium components is kept low if U.V. analysis is used (Setzkorn and Huddleston, 1965). U.V. measurement has been used for studying biodegradation of surfactants in continuous and semicontinuous activated sludge studies and in river die-away tests (Swisher, 1967b; Setzkorn and Huddleston, 1965). U.V. analysis usually fails to reveal minor modifications of the test compound since the U.V. absorption will often remain unchanged unless ring cleavage occurs.

e. Measurement of CO_2 Evolution: In the process of biodegradation, an organic molecule may be broken down to the ultimate end product, CO_2, and therefore, measurement of CO_2 evolution has frequently been used to follow biodegradation. A gas train assembly consisting of the reaction flask and CO_2 traps is generally used to measure CO_2 production (Atlas and Bartha, 1972, 1973; Sturm, 1973; Thompson and Duthie, 1968). The whole gas train arrangement may be mounted on a rotary shaker and agitated, but often it is not shaken. During the test CO_2-free air is bubbled through the test unit and the effluent gas is passed through the CO_2 absorbers (e.g., solution of barium hydroxide or potassium hydroxide). Absorbed CO_2 is determined by titration, or by radioassay if a radiolabelled test compound is used. Non-biological evolution of the gas is evaluated by aerating a sterile control with CO_2-free air. The quantity of CO_2 evolved may be compared with the theoretical maximum of CO_2 production determined by wet ashing of the test compound (Atlas & Bartha, 1972). In the interpretation of CO_2 production data, it must be remembered that, while oxidizing the test organic compound to carbon dioxide and water, microorganisms are also synthesizing new cell material from the compound. Measurement of CO_2 evolution is especially useful to measure ultimate biodegradability.

f. Oxygen Consumption: Oxygen consumption linked to the oxidation of the test chemicals can also be used to determine biodegradability of chemical compounds in the aquatic environment. Methods for measurement of oxygen include manometry, polarography and chemical methods, and these have been discussed in greater depth in Sec. III A.1, p. 51.

Oxygen consumption studies have been done both with mixed cultures (Nelson _et_ _al_., 1961; Hunter and Heukelekian, 1964; Blakenship and Piccolini, 1963) and with pure cultures of microorganisms (Heyman and Molof, 1967; Ellis _et_ _al_., 1957; Walker and Cooney, 1973a). Measurements of oxygen consumption linked to the microbial oxidation of the test compound is an indirect method for assessing biodegradability. The technique fails to reveal information about the nature of the intermediate compounds formed during degradation. The advantage of the technique, like CO_2 evolution, is that it can be used without developing an analytical method for the test compound. The extent of biodegradability is usually derived by comparison with theoretical oxygen demand for the test compound. In this comparison one must take into account the fact that, in most instances, biochemical oxygen demand is considerably lower than theoretically possible for complete oxidation since often 5-40% of the test carbon is used by microorganisms for synthesizing new cell material. When the test is run in the presence of external carbon source or when the endogenous respiration rates are higher, the interpretation of the results may be difficult because of the possible influence of the test compound on the oxidation of external carbon source or on endogenous respiration.

g. _Microbial Growth_: A simple test for determining the biodegradability of a chemical compound is to show microbial growth on that compound. A basal salt medium supplemented with the test compound is inoculated with the potential organism (commonly stocked organism or isolated by enrichment culture technique) and growth is measured by one of several methods; e.g., turbidity, cell count, dry weight determination (Prochazka and Payne, 1965; Payne and Feisal, 1963; Forsberg and Lindquist, 1967). Prochazka and Payne (1965) have shown a direct correlation between culture turbidity (a growth indicator) and degradation using mixtures of C_{10}-C_{20} secondary alcohol sulfates. The use of bacterial growth as an indicator of biodegradability can be applicable only for those compounds wihch are susceptible to extensive degradation and can support growth; i.e., serve as a source of carbon and energy for a bacterium. Therefore, this assay method has somewhat limited utility.

h. Bioassay: Bioassay methods involve exposing some type of living organisms to the test solution to measure the concentration of the test compound. One such method, but just one of very many, is described by Yasuno et al. (1966) for determination of organophosphorus insecticides. The test insect used for the biological assay was young 4th instar larvae of Culex pipiens. Some 30-50 larvae were added to 250 ml of the biodegradation test medium containing the insecticide and the results were read after 24 hours of exposure. Advantages and disadvantages of the bioassay technique are discussed in detail in Section IV B.2., p. 256.

i. Determination of Total Carbon: Measurement of total carbon concentration can be used to monitor the course of biodegradation. The methods for total carbon determination include chemical oxygen demand (COD) by dichromate method (APHA, 1971) and dry and wet combustion methods (Pickhardt et al, 1955, Weber and Morris, 1964). An alternate approach (combustion method) involves complete oxidation of the sample in a stream of air or oxygen which passes over a catalyst; the carbon dioxide produced may be measured by I.R. absorption (Van-Hall and Stenger, 1964) or by one of several other procedures.

If degradation of a mixture of chemical compounds, or the test organic compound in the presence of exogenous carbon is investigated, the method fails to provide a direct demonstration as to the extent of degradation or which materials in a sample are degraded. No information can be obtained by this method concerning the nature of the intermediates formed. The differences in the susceptibility of chemical compounds to combustion may sometimes introduce error in the results (especially with COD).

j. Others:

Surface Tension: This test has generally been used for surfactants and is based on their ability to reduce the surface tension of water. The measurement is made with an interfacial tensiometer (Allred et al., 1964; Huddleston and Allred, 1965; SDA, 1969). Blankenship and Piccolini (1963) have pointed out that surface tension can only be used as a qualitative measure in a biodegrading system because different surfactants (and perhaps their intermediate degradation products) may differ in their degree of lowering the surface tension.

Foam Threshold: Determination of the foaming potential is another method which has been used in biodegradability studies with surfactants. Foamability is determined by shaking a sample of the test medium in a glass stoppered graduated cylinder and reading the amount of the foam present (Huddleston and Allred, 1965; Lashen et al., 1966). The foam present can also be read by the foam test machine (Bacon, 1966). The method is not suitable for quantitative estimations and has only been used by workers in cases where chemical analysis is difficult.

Oil Dispersion: Oil dispersion has been used to assay for the microbial degradation of crude oil. Oil dispersion is measured by determining the turbidity of the reaction medium after vigorous shaking (Reisfeld et al., 1972). A sterile medium containing the crude oil is used as a blank.

D. Evaluation of the Techniques used for Determining Biodegradation of Chemicals in Natural Water Systems:

In evaluating the test methods which have been used for determining persistence of chemicals in the aquatic environment, a number of criteria can be successfully used. These include comparing the results obtained from a particular test method with those obtained by other test methods, wherever possible with the results of the field experiment and with the known environmental persistence of the compound as assessed from the monitoring data. Although any method can be evaluated for its ability to yield accurate qualitative as well as quantitative information, the data available in the literature thus far have permitted only qualitative comparison, e.g., whether a compound is shown to be biodegradable. A comparison based on the quantitative results, e.g., rates of biodegradation, obtained from different test methods, or a comparison of the degradation rates with known environmental persistence of a compound has generally not been attempted. This is perhaps because the quantitative results from different degradation test methods and from the environment have varied considerably, making it difficult to draw any conclusion about the actual rates of breakdown. The factors which affect biodegradation are numerous and have varied from test to test and from laboratory test to natural conditions. Prior to attempting to evaluate the test methods, it will perhaps be useful to discuss these factors to understand how and to what extent they are responsible for discrepancies in the degradation results.

1. Factors affecting biodegradation:

In the elimination of polluting substances from waters, the organisms primarily responsible for degradation are microorganisms. In most cases, the factors which influence the activity of these microorganisms will affect the biodegradation results. Some of the important variables pertinent to biodegradation are: type of microorganism, mineral salt composition, test chemical concentration, supplementary organic nutrients, O_2, temperature, pH, light, etc.

a. Type of inoculum:

Perhaps one of the most important factors that affects the results of a biodegradation test method is the nature and quantitiy of the microorganisms used to metabolize the test organic compound.

In biodegradation tests, the pure strain or a mixture of microorganisms has been used. Ordinarily natural microbial communities have been preferred, since populations may complement each other in their biochemical capabilities. Furthermore, it is unlikely that a single species of microorganisms would be able to catalyze biodegradation of so many structurally different molecules since the number of enzyme systems is limited in any one population. The work of Cook (1968) and many others has demonstrated that pure strains of microorganisms quite often are not as effective as a mixed community in biodegradation of organic compounds. Cook (1968) observed that with the pure cultures isolated from sewage effluent, degradation of the test detergent was considerably lower than was achieved by the mixed culture present in the effluent.

Several researchers (McKenna and Kallio, 1964; Painter, 1973a) have studied degradation of chemical compounds with a large number of pure cultures; the number of organisms that grow on the test chemical was used as biodegradability criteria. The results obtained from these large numbers of pure cultures may be considered somewhat similar to mixed culture.

The number of microorganisms initially present in the biodegradation test solution is generally not so important in terms of degradation, because of the short generation time for most microorganisms. Only the initial rate of degradation may be affected by the number of microorganisms. This point is evident from various studies (Eden et al., 1967; Garrison and Matson, 1964; Conway and Waggy, 1966) in which researchers have used river water fortified with added microorganisms to initiate rapid biodegradation.

The source of microorganisms can also be very important both in terms of the type of microorganisms that may be present as well as the number of each type. Swisher (1970) has found that the degradation of TBS (alkylbenzene sulfonate derived from tetrapropylene) was higher in the river die-away test than when examined by shake culture and batch or continuous activated sludge test. It should be emphasized here that this is not generally the case and may be true only for TBS. Since river water contains relatively few bacteria, the high degradation activity was attributed by Swisher (1970) to the natural species distribution in the river water. The source of microorganisms for testing biodegradation in the aquatic environment has generally been from such sources as sewage, activated sludge, river water, river or lake mud, and trickling filter slime. Many of these sources have been examined for their bacterial composition and have been found to be considerably different from each other. For example, activated sludge from varied sources was found to contain 72 different species classified in 14 genera (McKinney and Wetchlein, 1955). Hoadley and McCoy (1965) have reported isolation of 11 species of bacteria from the lakes and streams. Many researchers have not added any inoculum to their biodegradation test medium, and have relied on the organisms already present in the sample or those introduced from the atmosphere (Swisher, 1966).

Another very important parameter which should be considered while studying biodegradation of a chemical compound is the effect of microbial adaptation to the test chemical. From the biodegradability point of view, organic compounds can be 1. readily utilizable, 2. utilizable after acclimation, 3. slowly utilizable under all circumstances, or 4. not utilizable. For synthetic organic compounds which are not likely to be found in the environment, acclimation of organisms prior to studying degradation can be extremely important. The enrichment culture technique is a means of isolating or developing bacterial strains capable of degrading a particular organic compound. Importance of acclimation is indicated from the work of Huyser (1960), who reported that 5-6 days were needed for disappearance of 8-phenylpentadecane LAS in unacclimated river water but only 1-2 days in acclimated river water. Pfeil and Lee (1968) observed that no obvious degradation of NTA was catalyzed by nonacclimated seed organisms, whereas acclimated seed organisms were able to degrade NTA following a lag period of three days. Lashen et al (1966) observed rapid and extensive degradation of octylphenol ethoxylate in river water (from below heavily industrial urban area) bearing an acclimated microflora whereas very slow degradation was noticed in other river waters. Acclimation is sometimes unpredictable, and even after baceria have become acclimated to a chemical under one set of conditions, there is not guarantee of immediate action under other conditions. However, studies by Cook (1968) have indicated that acclimation may not always be required for biodegradation. She found that control cultures grown without surfactant in agar were just as effective as the "acclimated" ones. Others have made similar observations with different compounds.

b. Mineral Salt Composition:

For proper growth and function of microorganisms in the biodegradability test, elements such as nitrogen, sulfur, phosphorus, and magnesium at relatively high concentrations, and various other elements, such as iron, copper, manganese, and zinc, in trace amounts are required. Certain bacterial species may also require some preformed organic co-factors, e.g., certain vitamins. Hattingh (1963) has pointed out that the optimum feed should contain at least 5.3 parts of nitrogen and 1.2 parts of phosphorus for each 100 parts of BOD. Requirement for nitrogen and phosphorus supplement for biodegradation is also suggested from the studies of Atlas and Bartha (1973). These authors found that the sea water contains very low concentrations of nitrogen and phosphorus and, therefore, degradation of petroleum in the sea water is slow. When sea water was supplemented with these essential nutrients, degradation was stimulated several fold.

Biodegradation tests have generally been performed using the microorganisms in one of the following two phases: (1) Growing cells, and (2) Resting cells. In studies with growing cells, degradation of organic compound is linked to the growth of the organisms while in the case of resting cells, no increase in cell population usually occurs, and, therefore, degradation is the result of the preformed enzymes in the microorganisms. The composition of the medium used in the two types of studies has been considerably different. For example, resting cells are generally suspended in buffer alone (Focht and Joseph, 1971;

Focht and Williams, 1970), whereas with growing cells complete media have generally been used (Huddleston and Allred, 1963; Bunch and Chamber, 1967).

Several modifications in the mineral salt compositions have been made by researchers to facilitate the use of a particular analytical method. For example, Cordon et al. (1968) have used nutrient salt medium free of sulfate to permit following sulfate ion formation from anionic detergents.

In the literature, there are almost as many different mineral salt media reported as there are biodegradability studies, but there is no self-evident reason and probably no basis for this practice. This is mainly because each researcher has preferred his own recipe. No attempts have been made to determine if these different variations in the compositions of the mineral salt medium affect the biodegradability of a chemical compound, but the effect is probably small.

c. Test compound concentration:

The concentration of the test chemical initially added in the biodegradability test medium can affect the rate and/or the extent of biological degradation. The concentration of organic chemicals found in surface water is normally on the order of a few milligrams per liter or lower. Investigators have generally used much higher concentrations in biodegradation test methods for many reasons, including a limited sensitivity of the analytical method. For closer simulation of field concentrations, it may be desirable to use lower concentrations. An important phenomenon which is very much concentration dependent is acclimation of the bacterial cells. Unusually low substrate concentrations either fail to allow acclimation or require prolonged incubation periods.

Pfeil and Lee (1968) have investigated the biodegradation of NTA in aerobic systems at various concentrations. These investigators found no measurable degradation after 20 days incubation at NTA concentration of 5 mg/ℓ. However, in cultures where NTA concentration was 10 mg/ℓ, extensive degradation was initiated after 10 days of lag, possibly due to the development of some acclimated organisms. The upper limit for the concentration of the test chemical may be imposed by the toxic or inhibitory action of the chemical on the microorganisms or by a low solubility in water. Fuhrmann et al. (1964) observed a significant lengthening of the induction period and a lower reaction rate with an increase in concentration of the surfactant. These authors concluded that the inhibitory effect may be due either to bactericidal or bacteriostatic effects of the surfactant at higher concentration, and/or decrease in oxygen in water with increasing surfactant concentration. An increase in the lag period with increase in concentrations was also observed in the studies of Aly and El-Dib (1972) with the pesticide Baygon. The inhibitory effect of high chemical concentration is also clear from the studies of Baird et al. (1974) with phenols. Using Warburg respirometry, these researchers found that even at relatively low concentrations certain phenolic material produced deleterious effects on respiration. Although all the seven phenols tested were degraded 100% at 1 mg/ℓ concentrations, at higher concentration the degradation was severely inhibited.

d. Supplementary Nutrients:

Several researchers have studied biodegradation of organic compounds in the presence of a readily utilizable external carbon source.

Addition of a carbon source to the biodegradation test medium may be expected to enhance degradation in one of three ways:

1. The external carbon source may stimulate the growth of microorganisms responsible for breakdown of the test chemical so that the test vessel has a higher cell density.
2. It may induce enzyme systems which, in addition to catalyzing a reaction involving the natural substrates, may also attack the test compound.
3. It may provide energy for a reaction sequence involving an initial endergonic step.

The presence of a supplementary organic nutrient may permit cometabolic degradation for those chemicals which fail to serve as a sole source of carbon and energy for microorganisms. Cometabolism is a process where a non-growth substrate is metabolized by a microorganism needing a different compound as a carbon and energy source occurs. Horvath (1972a) has criticized test methods which have been used for demonstration of biodegradability but do not allow for possible cometabolic degradation. The effect of the presence of external carbon source on biodegradation of environmental pollutants has been investigated by many workers. Horvath and Koft (1972) have reported that although a branched chain ABS, tetrapropylene benzene sulfonate (TBS), failed to support growth of Pseudomonas, when glucose was added growth occurred and TBS was degraded. Decomposition of the herbicide 2,3,6-trichlorobenzoic acid by a Brevibacterium was reported to be accelerated by the addition of benzoic acid to the culture (Horvath, 1972b).

Addition of an external carbon source in many cases has been found to slow down or even stop degradation of the test chemicals. This has generally been attributed to the preferred metabolism of the external carbon source, or it is possible that the external carbon source may have a toxic effect on the biodegradation of the test chemical. Also, it is possible that populations using the added compound deplete the supply of some essential nutrient. Fuhrmann _et al_. (1964) observed that biodegradation of a straight chain ABS was delayed if an easily utilizable carbon source (Lactose broth) was added during the course of biodegradation. Ciattoni & Scardigno (1968) have reported the inhibitory action of glucose on biodegradation. In river water, 50 ppm of glucose delayed the onset of degradation of 10 ppm of LAS as long as glucose was replenished and maintained above 30 ppm. The inhibitory effect may be attributed to a reduction in the rate of synthesis of certain enzymes of LAS degradation by readily metabolizable carbon source such as glucose (the phenomenon is referred as catabolite repression, for details see Paigen and Williams, 1970).

Sikka and Saxena (1973) have compared the influence of two different types of external carbon sources on the degradation of herbicides endothall (1) glucose, which is a good source of carbon and energy, and (2) yeast extract, which serves predominantly as an exogenous source of carbon but also contains vitamins and other nutrients. Their findings indicated that while the presence of yeast extract stimulated the degradation of endothall, glucose caused a slight inhibition of degradation.

e. _Oxygen Requirement_:

For the oxidation of organic chemicals by microorganisms, a terminal electron acceptor is necessary. Dissolved molecular oxygen, under aerobic conditions, and nitrate, sulfate or carbon dioxide under anaerobic conditions can serve as acceptors. Extensive degradation of chemical compounds in the environment is, for the most part, an aerobic process (Maurer et al., 1971; Wayman and Robertson, 1963). However, replacement of an air stream with a stream of pure oxygen results in decreased degradation (Fuhrmann et al., 1964). This has been postulated to be due to the unavailability of CO_2 to microorganisms, but it could also be the result of O_2 toxicity.

Under anaerobic conditions, biodegradation is generally slower. However, some compounds are more susceptible to attack under anaerobic conditions. For example, Hill and McCarty (1967) have investigated aerobic and anaerobic degradation of chlorinated hydrocarbon pesticides. These investigators concluded that initial stages in the degradation were more rapid under anaerobic than under aerobic conditions for the compounds studied.

f. Temperature, pH, light, etc.:

The majority of the known biochemical reactions increase in rate with an increase in temperature up to a certain optimum temperature. Best growth of most bacteria is usually observed between 20 and 37°C. Proper temperature is also important for acclimation of microorganisms to a new substrate. It has been reported that at 5°C acclimation to a new chemical may take about five times as long as at 30°C (Water Pollution Research Laboratory, 1972). The effect of temperature on biodegradation by mixed populations such as those in rivers and activated sludge is further complicated since a change in temperature may give rise to a change in the species composition of the community.

Fuhrmann et al. (1964) have studied the degradation of straight chain ABS at various temperatures and found that essentially no degradation took place between 0 and 5°C. Such temperatures may be expected for 3-6 months of the year in many industrialized areas of the world. The above researchers also found that with an increase in temperature the acclimation period was shortened and the rate of breakdown increased. Similar observations concerning the effect of temperature were made by Evans et al. (1973), Evans and David (1974), and Atlas and Bartha (1972) for biodegradation of urea, ethylene glycols, and petroleum, respectively. The effect of temperature on biodegradation of chemical compounds in waste treatment system (Hawkes, 1963) and in respirometric test conditions (Montgomery, 1967) has been reviewed.

The pH of the biodegradation test medium is an important factor which significantly affects the biodegradation rates. Strongly acidic and basic pH can inhibit the development of microorganisms; most microorganisms thrive best not too far from neutrality (pH 7). Heukelekian and Gelman (1951) investigated the effect of initial pH on the oxidation of industrial wastes in respirometers and found that oxidation was most rapid in the pH range 6 to 8. Studies of Aly and El-Dib (1972) have indicated another role for pH of the test medium in biodegradation. These investigators found that carbamate insecticides, pyrolam and dimetilam resisted biological oxidation; however, the hydrolysis products formed under strong alkaline conditons could readily undergo biological degradation in natural waters. These findings suggest that the rate of chemical hydrolysis (which is pH dependent) may be a limiting factor in biodegradation of certain chemicals.

Light has rarely been shown to have a direct effect on microbial degradation of organic chemicals. It is possible, however, that in certain cases the photoproducts (see Section V, p. 283) rather than the original compound may be attacked more easily by microorganisms. For such compounds biological degradation in the presence of light may vary considerably.

Investigators have generally preferred to incubate biodegradation reaction medium in room light (Sweeney and Foote, 1964; Weaver and Coughlin, 1964), or in dark to prevent the growth of algae (Warren and Malec, 1972; Barstlap and Kortland, 1967). Under anaerobic degradation conditions, incubation in dark is particularly desirable to minimize the growth of algae since they can release oxygen into the system (Maurer *et al.*, 1971).

2. Comparison of Methods:

The physical and chemical parameters employed in testing biodegradability have varied from one test method to another. The importance of these parameters in affecting biodegradation results has been discussed earlier. These conditions may be more favorable for biodegradation in some test methods than in others. These variations between the test methods may introduce qualitative as well as quantitative differences in the biodegradability (rate and/or extent) of a test compound. In order to evaluate the internal consistency of the results obtained by various biodegradation test methods, some investigators have examined the fate of single chemical compounds by different test methods. Their studies are summarized in this section.

Swisher (1970) has compared various methods based on the results of primary degradation of tetrapropylene derived ABS (henceforth referred to as TBS). He found that the closed (dilution) bottle test and shake culture test hardly accomplished any degradation of TBS, whereas in the river die-away test it was often degraded to the extent of 65-75% (based upon loss of MBAS). The author concluded that although the bacterial concentration in river water is low, the higher biodegradability potential of this test must be due to the natural species distribution and the natural medium. Garrison and Matson (1964) determined the relative biodegradability of several classes of nonionic surfactants by shake flask, die-away and Warburg respirometry. Straight chain alkylphenol was found to be more biodegradable in either the shake flask or die-away tests than was the corresponding branched chain product. With branched chain alkylphenol, a somewhat greater amount of biodegradation was indicated in the die-away test than in shake flask test. In the respirometry test, none of the phenols or alkylphenol-straight or branched chain ethoxylates were significantly degraded. Direct comparison of bacteriological slant culture technqiue, river die-away test and shake culture test on various representatives of the ABS group of detergents was attempted by Cook (1968). Relatively slow degradation was indicated for the slant culture technique in comparison to the other techniques. River die-away and shake culture test in this study gave more or less comparable results.

There are no reports in which attempts have been made to compare the results from model ecosystems to those obtained with other biodegradation test methods. Certain chemicals which have been tested in Metcalf's ecosystem have also been investigated by certain other test methods by some investigators.

and a comparison is possible in those instances. The extraordinary environmental stability of organochlorine pesticides dieldrin and DDT is shown in the Metcalf system by very low overall biodegradability index values* (0.00094 for dieldrin and 0.04031 for DDT). Eichelberger and Lichtenberg (1971) studied the persistence of the above organochlorine pesticides in river water and, similar to Metcalf's findings, reported no measurable degradation or chemical change. Comparison of the results from Metcalf's ecosystem to those obtained with other test methods for certain water-soluble compounds is somewhat more difficult. For example, benzoic acid is stored in the tissues of various organisms in substantial quantities and is perhaps not available for microbial attack, while aniline is not stored in appreciable quantities (Metcalf and Lu, 1973) and can be degraded by microorganisms. Distribution of benzoic acid and degradation products in the ecosystem (Metcalf and Lu, 1973) indicated that approximately 75% of the total ^{14}C in benzoic acid was present in the nonpolar forms which suggested (according to Metcalf's definition of biodegradability) a persistent nature of benzoic acid (overall biodegradability index in Metcalf's system = 0.335). Aniline, another water-soluble compound, which is not stored in significant quantities in food chains, was extensively degraded in the ecosystem (only 20% of the total ^{14}C in the nonpolar form, overall biodegradability index = 3.8). Both these compounds have been shown to be extremely biodegradable by other test methods (Buzzell et al., 1968, 1969). From this comparison, it appears that ecosystems, which have a tendency to store some compounds (see page 35) and thereby make them unavailable for microbial attack, may give misleading results concerning environmental persistence in the case of certain compounds.

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* Metcalf and Lu (1973) defined the biodegradability index as the polar products in the organism/non-polar products. For comparison purposes, we have calculated the overall biodegradability index which is the total polar products/total non-polar products.

3. Correlation between laboratory and field results:

The laboratory techniques used for studying biodegradation in the aquatic environment have generally attempted to retain as many important factors of the normal aquatic environment as possible. These factors are, however, so numerous and their influences so interwoven that any laboratory test method may be unable to account for all these parameters. In spite of these shortcomings, suitable test methods have provided information which can be extrapolated to the natural conditions. The ultimate evaluation for a test method is a comparison of its results to natural environmental results.

There has generally been good agreement between laboratory biodegradation test results and environmental persistence for compounds which are known to be extremely resistant to biodegradation. For example, DDT, which is known to persist in the environment as shown by its widespread occurrence (Edwards, 1973) has been found to be persistent when tested by any of the several biodegradation test methods (Model ecosystem, Metcalf *et al*., 1973b; River water test, Eichelberger and Lichtenberg, 1971; Modified Bunch and Chambers test, Leigh, 1969; Shake culture test using microorganisms from sewage lagoon, Halvorson *et al*., 1971). A substantial amount of monitoring data is now available on various isomers of PCB and, therefore, this group of chemicals can also be used as an example for comparison. Veith (1972) has found that the proportion of higher chlorinated isomers increases downstream in some Wisconsin rivers, whereas the lower chlorinated isomers disappear. Nisbet and Sarofim (1972) proposed that, since it is unlikely that the lower isomers are differentially retained in the sediments (Veith, 1972; Nisbet and Sarofim, 1972), their disappearance probably is due to their rapid microbial decomposition in the river environment. The only laboratory study which has investigated the relative biodegradability of various isomers of PCB was done by Metcalf and Lu (1973) in the model ecosystem. A comparison of environmental behavior of trichloro-, tetrachloro- and pentachlorobiphenyl in the model ecosystem (Metcalf and Lu, 1973) showed a decrease in the overall biodegradability index with increasing chlorination (BI = 0.1093 for trichloro-, 0.0606 for tetrachloro-, and 0.02366 for pentachlorobiphenyl). These results are in agreement with the suggested environmental behavior of PCB isomers discussed above. Kaiser and Wong (1974) have investigated the degradation of Aroclor 1242 (mixture of PCB's containing mainly monochloro-, dichloro-, trichloro-, and tetrachlorobiphenyl) in shake cultures inoculated with pure cultures of bacteria enriched on Aroclor 1242. These researchers have also found that less chlorinated biphenyls are degraded preferentially.

Some other compounds have been studied in enough depth that they can be used to evaluate some of the test methods. Frank (1966) has investigated the fate of monouron in simulated ponds; their results indicate that the pesticide applied at the rate of 40 kg/ha persisted in excess of 2 years. In the river die-away test, however, monouron did not persist in significant concentrations after 4 weeks (Eichelberger and Lichtenberg, 1971).

Sikka and Rice (1973) studied the persistence of the herbicide endothall (7-oxybicyclo[2.2.1]heptane-2,3-dicarboxylic acid) in a farm pond. They reported that endothall could not be detected in the farm pond) top one inch of hydrosoil or pond water) after 30 days. Since endothall is water soluble and its loss due to volatilization is negligible, it was assumed that loss of endothall was due to biodegradation. In parallel aquarium studies, these researchers observed a several-fold higher rate of degradation of endothall. The aquarium studies were conducted using water and hydrosoil from a pond which had been previously treated with the test chemical and, therefore, the microorganisms probably had been acclimated. That endothall is rapidly degraded by microorganisms was also shown in a shake culture test inoculated with a species of Arthrobacter isolated by enrichment culture technique (Sikka and Saxena, 1973). Oxygen uptake studies with the Warburg method indicated that resting microbial cells oxidized endothall vigorously and at a linear rate (Jensen, 1964). The final oxygen uptake value, however, corresponded to only 37-43% of the theoretical oxygen demand of endothall. These results are difficult to interpret since Jensen (1964) in his experiments observed only 9-14% of the theoretical oxygen demand of glucose.

Urban et al. (1965) have studied the persistence of LAS and TBS in a 6.6 mile canal with a flow-through time of 6 hours. The results indicated 35% degradation (as revealed by the loss of methylene blue activity) during passage of LAS compared to 15% for TBS. When the retention time was increased to 10 days, LAS was degraded about 100% compared to 22-35% for TBS. That LAS was more biodegradable than TBS is also supported from the monitoring data of Merrell et al. (1967) as discussed below. In 1961, at Santee, Calif., the influent of the treatment averaged about 16.5 ppm in MBAS, the effluent 8.5 ppm. It was down to 6.7 ppm after the oxidation pond. Upon change from TBS to LAS by the U.S. detergent industry, the oxidation pond effluent dropped to about 1 ppm, suggesting rapid degradation of LAS.

The biodegradation of TBS (near C_{12} alkyl group) and LAS (mixed chain lengths) has also been tested by various laboratory test methods by a number of investigators and the results are summarized in Table 16.

Table 16. Comparison of Biodegradation of LAS and TBS by Different Experimental Techniques
(Taken from Swisher, 1970)

Substrate: LAS

Method	Extent of Degradation %	Time of Incubation (Days)	Reference
River die-away test*	97	30	Knaggs *et al*., 1965
" " "	100	7	Lang *et al*., 1965
" " "	100	9	Wayman and Robertson, 1963
Shake flask*	90-92	4	Tomiyama *et al*., 1969
" "	91-96	-	Oba *et al*., 1968
" "	100	8	Lang *et al*., 1965
BOD	19**	7	Krone and Schneider, 1968

Substrate: TBS

Method	Extent of Degradation %	Time of Incubation (Days)	Reference
River die-away test*	27	30	Berger, 1964
" " "	25	32	Renn, 1964
" " "	30	30	Knaggs *et al*., 1965
Shake flask*	17-20	4	Renn, 1964
" "	18	5	Allred *et al*., 1964
BOD	2**	5	Ryckman, 1956, Ryckman and Sawyer, 1957
"	5**	22	Ryckman, 1956

* Analytical Method: Loss of methylene blue activity

** Percent of that calculated for complete oxidation of the substrate

Since LAS and TBS are crude mixtures and their composition can vary somewhat, the results should only be used to derive qualitative conclusions. The laboratory shake culture and river die-away test results appear to be in agreement with the field results and monitoring data. These tests have also revealed approximately 18-30% biodegradability of TBS and 90-100% of LAS. Since the degradation criteria in the river die-away and the shake flask method is the loss of the parent compound, the data from the above tests can not be compared with BOD data.

Although it is extremely valuable to know the rates at which a chemical will degrade in the natural environment, extrapolating the laboratory biodegradation rates to the rate of breakdown in the natural aquatic system has not been very successful.

4. General Discussion of Various Test Methods:

The formulation of a meaningful method for testing biodegradation of organic chemicals involves the selection of (1) an appropriate test environment (or environments) with control of the experimental variables, and (2) a suitable means of following the biodegradation. This literature review has revealed a number of test methods for assessing biodegradability in the aquatic environment. These have varied from quite complicated techniques such as the "model ecosystem" of Metcalf et al. (1971) or the aquatic ecosystem at EPA Athens laboratory, to relatively simple techniques, such as BOD.

In many cases, researchers have attempted to simulate to a degree the natural environment. The simulation has involved adjustment of the parameters such as microbial concentration, diversity of the microbial community, external carbon and energy source, oxygen, nutrient concentration, etc. A particular test method may be subject to other problems besides failing to simluate the natural environmental conditions, such as inconvenience of procedure, time requirements, equipment or lack of reproducibility. The test methods are compared in this section from the point of view of these parameters. The methods described in the literature can be suitable for: (1) preliminary screening for biodegradability, and (2) determination of intermediate metabolites and the routes of breakdown. These two categories of biodegradation test methods are evaluated separately.

a. Rapid Screening Test for Biodegradability:

In evaluation of the environmental persistence of a chemical compound, it appears logical to first run a rapid test to screen for biodegradability. The preliminary screening test may reveal if it is necessary to detect the metabolites formed during degradation. For example, if the BOD for a compound is close to the theoretical oxygen demand calculated for complete oxidation, the question of accumulation of any undesirable metabolites is less likely to arise. A number of screening tests have been used by researchers to obtain information about overall biodegradability. Most researchers have preferred using natural mixed cultures of biological material in these tests because they are readily available and the results can be extrapolated to natural conditions with less difficulty. Biodegradability tests such as biological oxygen demand, river die-away test, shake culture test using mixed inoculum, and perhaps more complex model ecosystems could qualify for this category.

Biochemical Oxygen Demand:

Biochemical oxygen demand, whether determined by dilution method or by respirometry, is an indirect test for assessing biodegradability and does not provide any information about the nature of the degradation products. However, the method requires no specific analytical method for estimation of the chemical compound and, therefore, the test is relatively simple. Since the procedure measures oxygen consumption, there can be little confusion due to physical absorption of the test chemical.

Since oxygen is utilized by microorganisms for a multitude of complex metabolic reactions and not simply for oxidation of the test chemical, interpretation of oxygen consumption data is sometimes difficult. A portion of the test chemical may be used for the synthesis of cell materials which is much less oxidized than ultimate degradation products CO_2 and H_2O. Because of uncertainties, oxygen uptake methods would, at best, give only qualitative results. The problem of interpreting the oxygen uptake data is further magnified due to possible changes in the endogenous oxygen uptake rate (oxygen uptake in the absence of test chemical) of microorganisms, which is generally subtracted from the oxygen uptake rates obtained in the presence of the test chemical. It is possible that the presence of test chemical may cause a considerable change in the endogenous respiration rate and, therefore, subtraction of endogenous rate will not be representative of the oxygen used for biodegradation of the test compound. The problem can be overcome by adjusting the inoculum to give very little or no endogenous oxygen uptake.

If oxygen uptake is measured in the presence of an exogenous carbon source (other than the test chemical), the exogenous source could be subject to oxidation, confusing the test. In extreme cases, the test compound may stimulate the oxidation of exogenously added carbon source without being degraded itself. Alternatively, the test compound may inhibit the oxidation of exogenously added carbon and give misleading results. Error can also be introduced in oxygen uptake data if oxygen is also being consumed for nitrification processes. This will lead to erroneously high oxygen consumption rates. This problem can be easily overcome by determination of NO_3^- formed in the medium (see Buzzell et al., 1968).

If the investigator desires, samples can be removed during the course of oxidation to correlate the oxygen utilization curve with other analytical data. Realizing that the oxygen utilization data alone may be inadequate to help in understanding the behavior of organic chemicals in the aquatic environment, Buzzell et al. (1968) followed the carbon removal, changes in bacterial population and nitrification in the samples removed for BOD assay. The carbon removal data normally support the oxygen data and indicates the completeness of biodegradation; bacterial enumeration measurements are of value in determining both biodegradation and toxicity of the organic chemical, and measurement of nitrification gives data concerning noncarbonaceous oxygen utilization which is corrected from the total oxygen consumed. According to these researchers, the BOD test method can thus be made more truly quantitative and allow wider applications.

Oxygen uptake measurements have been performed by dilution method or by respirometric methods. The respirometric method is generally considered more precise as far as measurement of oxygen demand is concerned. Both methods, however, have the same interpretation problem as discussed above. The microbial concentration used in respirometric method is higher and thus it simulates treatment plant conditions more closely than does the dilution method. The respirometric tests are normally less time consuming than the 5-day dilution test. Among the respirometric methods, polarographic methods, e.g., oxygen sensing electrodes, are generally preferred over manometric methods for measurement of oxygen since the latter method is more time consuming and less sensitive.

River Die-away Test:

This is one of the earliest tests used to measure biodegradability of chemical compounds. The reaction conditions in this test more closely approach the conditions encountered in nature. The test is simple to run and requires a minimum of equipment. However, one of the more serious shortcomings of the test is the variation in bacterial count and composition between different rivers, between different points in the same river, and even at the same point at different times in a given river. Considerable variations in the bacterial count in river water will also be introduced due to seasonal variations. The river water from different sources may also vary in the concentration of the nutrients and toxins present. These fluctuations may cause variations in the results between laboratories studying the biodegradability of the same material. In addition, the low concentrations of microorganisms in river water have resulted, in some cases, in long periods of incubation before noticeable degradation.

Rivers in many urban industrial areas may contain organisms which are already acclimated to several synthetic chemicals. River water from such sources may exhibit rapid degradation rates while river water from those rivers which receive very little or no organic chemicals may require a long acclimation period. Lashen et al. (1966) have investigated the role of natural acclimation of river microorganisms in biodegradation. They reported that samples from the Schuylkill, Delaware and Ohio Rivers below heavily industrial urban areas show a rapid and extensive degradation of octylphenol ethoxylate. River water from the Schuylkill, Delaware and Ohio rivers taken from areas where usage of octyl- and nonlyphenol ethoxylates is estimated to be very low, on the other hand, gave slower rates of degradation.

The low organic nutrient levels and low solids content of the river water system may facilitate isolation and analysis of the test chemical. On the other hand, the variations in the composition of the river water may introduce variability in the extraction procedure and/or quantitative measurement.

The various modifications made in the river die-away test include the use of fortified and inoculated water, or polluted water. Although these modifications have eliminated the need for prolonged incubations, the variations in the river water would still be a problem. Furthermore, with the introduction of exogenous material in the natural river water, the extrapolation of the results to natural conditions will be more difficult. The anaerobic and microaerophilic river die-away test is a useful test for assessing biodegradability in systems containing no oxygen or low concentrations of it. Such conditions are likely to occur in places such as the deeper sediments of rivers and lakes.

Shake Cultures Inoculated with Natural Communities of Microorganisms:

Shake culture test is a rapid test designed primarily to assess biodegradability of organic compounds under aerobic conditions. The test has been accepted as a screening or presumptive test for biodegradability by the Soap and Detergent Association (1965) Subcommittee on Biodegradation Test Methods. The test generally uses high concentrations of microorganisms; subsequently, the duration of the test is relatively short. The medium used for the test is of defined composition resulting in good reproducibility. The test can be run in the presence or absence of an external carbon source, the latter conditions permitting the occurrence of cometabolic degradation. The medium used in the test is most often specially formulated and contains all the essential nutrients required for proper growth and function of many microorganisms and provides opportunity for biodegradation. The conditions in this method may be much more favorable for degradation than generally encountered in the natural environment. Subsequently, extrapolation of the results to natural conditions may be difficult. Most researchers have preferred to use acclimated seed to obtain rapid degradation. Under natural conditions, however, unless a particular compound is continuously released into the environment in sufficient quantities, such an acclimation may be quite slow. However, the use of acclimated seed is desirable in that the rate of biodegradation under idealized conditions is obtained. In actual practice, more complete information about biodegradation is obtained if the test is run with both acclimated and unacclimated seed. Acclimation of the seed is generally carried out by frequent subculturing of the seed in fresh medium containing the test compound (Huddleston and Allred, 1963; Bunch and Chambers, 1967). When acclimating a seed to a chemical which is toxic to the biodegrading populations, the preferred technique is to acclimate the seed organism in slowly increasing concentrations of the test chemical (Schwartz, 1967; Hemmet, 1972).

The source of microbial inoculum in the shake culture test has varied considerably. Researchers have used microorganisms obtained from sources such as sewage, lagoons, lake sediments, etc. Sewage microorganisms have been used most frequently. The use of unidentified and nonspecific mixed cultures in a test may always be criticized as an unknown variable and may never give desired confidence to the test. Swisher (1966) used as inoculum the microorganisms which had developed in an uninoculated test medium.

Inoculum developed in this manner may vary from one laboratory to another. In an attempt to minimize the variations in the type of inoculum used by different laboratories, several researchers have attempted to preserve the seed so that the same seed can be made available to other laboratories for testing biodegradability. Some researchers, however, feel that it is unnecessary because the biological composition of the natural seed (e.g., sewage) obtained from various sources does not vary significantly (Swisher, Alexander, personal communication). Furthermore, subjecting seed to preservation (e.g., lyophilization, air drying) may cause loss of activity. The slant culture technique was evaluated by Cook (1968) and found not suitable for determining biodegradability. The technique gave variable results which were not reproducible and the degradation achieved was very low in comparison with that achieved by other methods.

Model Ecosystems:

Model ecosystems used by researchers to date are oriented more towards answering questions concerning bioaccumulation of the chemical compounds than environmental persistence or biodegradation. Most of these systems are not well defined in terms of their microbial composition and, therefore, their reproducibility and subsequently their use in environmental persistence studies is uncertain. However, the metabolic fate in the food chain is quite reproducible in these systems. In Metcalf's ecosystem, the source for microorganisms is old aquarium water; the microbial composition of aquarium water can vary considerably from one laboratory to another and from day to day in the same laboratory. In other ecosystem studies the source of microorganisms is not specified, although some microorganisms, e.g., those associated with the food chain organisms or present in the aqueous medium are undoubtedly present in the ecosystems.

Biodegradability in these studies generally refers to the breakdown occurring in the food chain organisms rather than in the environment. For instance, the biodegradability index (polar products in organisms/nonpolar products) in Metcalf's studies is calculated for fish and for snails. Since Metcalf's system measures biodegradability in terms of the ratio of polar and nonpolar metabolites, it assumes that conversion of a compound to a polar metabolite is an indication of biodegradability. Although this may be quite often true (see Section VIII, p. 461) there are examples where a compound is water soluble and persistent. For example, a portion of the soluble organic carbon of the oceans is not attacked by microorganisms (Alexander, 1973a).

As pointed out earlier in Section II,(p.33) the role played by unicellular microorganisms in the overall environmental persistence of the chemical compound seems far more important than that exhibited by organisms at higher tropic levels. Alexander (personal communication) feels that for testing biodegradability alone, it is unnecessary to use a complex ecosystem which contains the total aquatic food chain. Food chain organisms at higher trophic levels (henceforth referred to as higher organisms) and microorganisms are generally considered widely different in their catabolic capabilities. Chemical compounds can be categorized into the following classes, depending upon whether they are degraded by microorganisms and/or higher organisms:

1. Compounds which are degraded by higher organisms as well as by microorganisms.
2. Compounds degraded predominantly by microbial action.
3. Compounds degraded predominantly by higher organisms. There are apparently no known examples in the class.
4. Compounds not degraded at all.

Model ecosystem studies have generally provided excellent information concerning environmental fate of compounds falling in Class 1 and Class 4. This is predominantly because the response of higher organisms and microorganisms is generally similar if a compound is highly biodegradable (e.g. glucose) or if the compound is highly persistent (e.g., chlorinated-hydrocarbon pesticides). Sufficient numbers of compounds falling in Group 2 have not been evaluated by model ecosystem studies and, therefore, universality of the application of model ecosystem technology in determination of environmental fate of chemical compounds is hard to assess. Benzoic acid provides an example of a compound which is not known to be environmentally persistent as shown by several test methods (Buzzell *et al.*, 1968, 69) but is persistent in the higher organisms (Metcalf and Lu, 1973) due to its ability to be stored in the higher organisms or conjugated.

The terrestrial phase in the Metcalf ecosystem is prepared from washed white quartz sand which is molded into a sloping surface. The problems associated with sand include (1) microorganisms associated with soil are absent, (2) failure to account for the effect of highly important parameters such as adsorption or binding of the chemical to the soil and reactions with soil colloids. These drawbacks have been addressed in the ecosystem studies of Isensee and Jones (1974) who have added soil to their ecosystem. In an effort to simulate erosion of pesticides on soil, they adsorbed the compound on soil first and then placed it in an aquarium and added water.

Metcalf's ecosystem also fails to permit sufficient rates of photodecomposition, contrary to what has been claimed. Their aquarium units are housed in environmental plant-growth chambers; the light source used in these chambers (combination of fluorescent and incandescent lamps) provides only a small fraction of the 290-350 nm light present in the normal sunlight (see Fig. 2, p. 43). The intensity of this effective component of U.V. light is further reduced in their system when the chamber is covered with plexiglas. As shown in Table 39 (p.343), plexiglass is capable of absorbing significant portions of ultraviolet of wavelengths less than 350 nm. The importance of simulation of sunlight both in terms of wavelength and intensity can be quite important with certain compounds. Isensee (1974) has found in his model ecosystem studies with dinitroaniline that exposure of the aquariums to various light conditions (e.g., white fluorescent light, subdued sunlight and direct sunlight) can result in a direct relationship between sunlight and dinitroaniline bioaccumulation. The bioaccumulation index follows closely the order of increasing ultraviolet content and increased intensity; i.e., white fluorescent light > subdued sunlight > less subdued sunlight > direct sunlight.

In summary, model ecosystems are more suitable for evaluation of metabolism by higher organisms and bioaccumulation through food chain organisms than biodegradation. If the radiolabelled test chemical is not available from a commercial source, it will have to be custom synthesized by a commercial firm or prepared in the laboratory which could be both expensive and time consuming. Model ecosystem studies of Metcalf, Isensee and others have provided excellent information regarding distribution and metabolic fate of a large number of environmental pollutants in higher organisms.

b. Biodegradation Test Methods for Determination of the Routes of Degradation

The screening tests discussed in the earlier section can only provide results concerning biodegradability of a test chemical. The value of the test will also be very much dependent on the analytical method which has been used for assessing biodegradation. For example, if the disappearance of the parent compound is the only parameter monitored, nothing will be revealed about the identities of the intermediate products of biodegradation. On the other hand, if a compound can be shown to provide total theoretical amounts of CO_2 to obtain biochemical oxygen demand approaching theoretical, it can be fairly safely assumed that the compound will be biodegraded to innocuous material, unless small quantities of highly potent toxins accumulate (Ayanaba and Alexander, 1974; Alexander, 1974). In reality, however, such clear cut results are not encountered too regularly. Therefore, it becomes essential to elucidate the identities of intermediate metabolites since they may be equally or even more toxic than the parent compound. Moreover, certain metabolites may persist for long periods of time under one set of conditions whereas they may be short lived intermediates of a metabolic pathway under others.

A detailed study of specific metabolic reactions is generally quite time consuming and requires a well equipped laboratory and, therefore, such detailed studies should be attempted only if preliminary evidence concerning transformation of the chemical has been obtained. The use of unidentified and nonspecific mixed culture in determination of pathways of degradation may always be criticized as an unknown variable which may not give reproducible results. However, if a researcher is simply interested in identifying persistent and toxic metabolites which may be formed during degradation, purification of a particular microorganism is often unwise. The disadvantage in using natural sources of mixed culture, e.g., sewage sludge, lake sediment, etc., is frequently that the metabolite may get bound to the particulate matter and may escape detection. Extraction of the adsorbed metabolite by drastic means may sometimes alter the nature of the metabolite (Chesters et al., 1974). Still, this is the model of the natural ecosystem, and the problems of extraction are often easily overcome. Although identification of persistent metabolites is also possible using Metcalf's ecosystem, the metabolites formed in this system refer to the breakdown in the food chain organisms rather than in the microorganisms. For this reason Metcalf's approach will not be elaborated in this section.

The information concerning the routes of degradation of chemical compounds has been derived largely from pure culture studies. Although very reproducible, the problem associated with pure culture studies sometimes is that they fail to account for breakdown of a compound by the combined action of two or more microorganisms or for one species providing growth factors needed by the degrading population. The importance of synergistic relationships in the degradation of chemical compounds has been demonstrated by Gunner and Zuckerman (1968). It is evident, therefore, that in certain cases pure culture studies may show the accumulation of a toxic metabolite, which in the mixed culture studies may have been decomposed by another microorganism. Alternatively, intermediates may accumulate in nature but not be found in a given pure culture, as is known for certain metabolic sequences (e.g. see Alexander 1972). The origin of pure cultures of microorganisms for degradation work has varied considerably. Pure cultures have been obtained from commercial sources, or isolated or enriched from natural sources. Enrichment of pure cultures using the test chemical as the sole source of carbon is far more commonly used. This procedure is normally less time consuming than screening a large number of microorganisms for their ability to degrade a particular test chemical.

Determination of catabolic pathways has been aided by the use of radiotracers. This is because the detection sensitivity of radiotracer techniques far exceeds the chemical and physical methods and because radiotracer studies provide a total accounting or balance sheet of the fate of the compound. Furthermore, the use of radiolabelled compounds allows the researcher to distinguish between the carbon provided by the degrading compound as opposed to cellular carbon from other sources. Also the technique affords the opportunity of following the fate of the test molecule within the cell. The use of radiotracers in studies concerning biological fate of chemicals has been discussed in a number of reviews (Casida, 1969; Kamen, 1957).

Elucidation of the pathways of degradation involves (i) identification of the degradative intermediates and (ii) assigning places to the intermediates in the scheme of degradation. The former is, however, more important from the environmental standpoint. The study of metabolic pathways begins with the intact cell and continues with fractionating of the cell into smaller units. Some limited information can be obtained by incubating the chosen microorganisms with the test chemical and then analyzing the culture fluids for the products of degradation. This approach was used by Alexander (1972) in determining an intermediate in the degradation pathway of 2,4-D. The appearance of 2,4-dichlorophenol in the culture fluid during the growth of Arthrobacter on 2,4-D, suggested the phenol as an intermediate metabolite. Chemical analysis of the culture medium of bacteria growing on α-naphthol has revealed information about the pathway of 1-naphthol degradation (Bollag et al., 1975). Frenne, et al. (1973) have used this procedure to elucidate metabolic pathways of pyrazon degradation.

The compound which accumulates in the culture fluid, however, need not necessarily be a degradative intermediate. Dagley and Chapman (1971) have stated that the easier it is to isolate a compound from metabolic fluids, the greater the caution to be exercised before it can be assigned the status of a degradative intermediate. A metabolite which accumulates in copious amounts may be suspected to be formed from a side pathway. Alternatively, it may be the end product of metabolism.

Quite commonly, intermediates remain within the cell, and thus have to be extracted out from the cell prior to their characterization. Considering this possibility, a number of researchers have extracted the cells as well as the culture fluid. Sikka and Saxena (1973) subjected the cell suspensions to hot methanol treatment in order to release the intermediates of endothall metabolism. Miyazaki, et al. (1970) in their metabolism studies deproteinized the cells by trichloroacetic acid to facilitate leakage; this was followed by extraction of the cells with ether.

Chemical intermediates generally are only present in the cell in minute concentrations which makes their extraction and identification sometimes difficult. An increase in concentration of the chemical intermediate will take place until the time that the rate of degradation equals or exceeds the rate of formation of the intermediate. A metabolite can be made to accumulate if the reaction sequence can be broken at a specific point. This can be accomplished either by inhibiting an enzyme or by adding a trapping agent that combines with the reaction intermediate.

Tiedje, et al. (1973) have used malonate (inhibitor of enzyme succinic dehydrogenase), arsenite (inhibitor of dehydrogenases containing dithiols, particularly α-ketoglutanic dehydrogenases, see Dawson, et al., 1959), and 2,4-dinitrophenol (uncoupler of oxidative phosphorylation), in an attempt to accumulate intermediates of NTA degradation. Fluoroacetic acid, an inhibitor of the tricarboxylic acid cycle, was used by Sikka and Saxena (1973) to promote the accumulation of an intermediate of the degradation pathway of the herbicide endothall. When inhibitors are used in intact cell studies, caution must be exercised in interpreting the results because the permeability barrier of the cell may sometimes prevent the inhibitor from gaining access within the cell.

Further evidence for the status of a compound as an intermediate in the pathway can be obtained by the technique of sequential induction. The concept is based on Stanier's theory (Stanier, 1947) which states that cells growing on a specific compound are sequentially induced to oxidize the intermediate oxidation products. For example, when a cell grows at the expense of compound A, it will contain enzymes that catalyze the breakdown of reaction intermediates B, C and D in the sequence. For example, Alexander (1972) and MacRae & Alexander (1963) observed that growth of a Flavobacterium on dichlorophenoxybutyrate resulted in the induction of enzymes capable of oxidizing not only the growth substrate but also 2,4-dichlorophenol, which suggested that 2,4-dichlorophenol was an intermediate in the pathway. The 2,4-dichlorophenoxyacetic acid (2,4-D) molecule (which would have been formed if phenoxybutyrate underwent beta-oxidation) was eliminated as an intermediate because dichlorophenoxybutyrate-grown cells failed to oxidize 2,4-D. This same approach has also been used to elucidate the pathways of breakdown of 2,4-D (Loos et al., 1967), 2-alkylalkanoic acids (Lijmbach and Brinkhuis, 1973), and primary alkylbenzene sulfonate and linear alkylated sulfonate (Cohn, et al., 1953). A limitation of the technique is that certain enzymes may be nonspecific and may catalyze the oxidation of compounds which may not even be part of the pathway. Moreover, sometimes a true intermediate may not be oxidized because it is unable to enter the cell. These and other limitations of this approach are discussed by Stanier (1947) and by Dagley and Chapman (1971).

Elucidation of biodegradation pathways with intact cells has sometimes been handicapped due to (1) difficulty in manipulating physical and chemical parameters (for example the permeability barrier of the cell prevents the investigator from removing any coenzymes, in an attempt to promote accumulation of an intermediate), (2) rapid reactions that do not allow examination of intermediate metabolites, (3) the difficulty in studying enzymes involved in degradation separately. Due to these difficulties, many researchers have used cell-free extracts prepared from the microorganisms for advanced degradation work. The major disadvantage in the use of cell-free extracts is the difficulty in extrapolation of the results to the natural environment. Disruption could result in interference of the degradation of the parent compound or of some of its metabolites due to inactivation of some essential enzyme(s), or in some other way change or introduce artifacts in the metabolic process in the intact cell.

Information on the metabolic pathways has been derived from cell-free extracts by breaking the reaction links at different points. This permits the investigator to examine the reaction sequence in small segments. Reaction links can be broken by inactivation of a particular enzyme by inhibitors, heat treatment, removal of coenzyme by dialysis, or fractionation of crude extracts by a method which removes one of the enzymes and leaves the others in a functional state. Some of these techniques have been used by Alexander and his associates (Bolleg _et_ _al_., 1968; Alexander, 1972) in elucidating the pathways of breakdown of 2,4-D. For example, in order to identify the products formed from phenol (an intermediate metabolite of 2,4-D metabolism) by the cell-free extracts of Arthrobacter, these researchers separated the phenol metabolizing enzyme from the next enzyme in the sequence by passing the cell-extracts through a Sephadex G-200 column.

Another approach which has been used for studying metabolic pathways is to determine if the cell-free extracts metabolize the postulated intermediates at a significant rate. In order for a compound to be a catabolite in the proposed sequence, it must be metabolized at rates that are compatible with the overall rate of degradation. In studies of 2,4-D metabolism, Loos, et al. (1967) found that the enzyme preparations converting the phenoxy compound to phenol, failed to metabolize 2,4-dichloroanisole at a significant rate. These results suggested that 2,4-dichloroanisole was not an intermediate in the 2,4-D metabolism.

The demonstration of appropriate enzymes in the cell-free extracts and/or their purification is also a step in the direction of establishing metabolic pathways. Ornston and Stanier (1966), by purifying the enzymes concerned with the degradation of benzoic acid and p-hydroxybenzoic acid, have shown that the metabolic routes for the two compounds are entirely separate and distinct. In view of the non-specific nature of a number of enzymes heavy reliance is generally not placed on this approach. Furthermore, the inability to demonstrate the presence of a particular enzyme is of little importance in eliminating a pathway, since enzymes can be inactivated during preparation of the extracts.

From the studies reported so far, it appears that once a pure culture of microorganisms that metabolizes the test organic compound has been obtained and cell-free extracts prepared, the techniques which are used for elucidating metabolic pathways are similar to those which have been used by microbial physiologists for studying metabolic pathways of a natural substrate (Dagley and Chapman, 1971). The application of these basic techniques in studying metabolism of environmental contaminants is well illustrated by studies on 2,4-D, which is one of the few synthetic organic chemicals of environmental significance for which degradation pathways have been reasonably well worked out (Alexander, 1972). Although the pathways established in pure cultures of microorganisms and/or in cell-free extracts may not be easily extrapolated to natural conditions, Alexander (1972) has stated that such studies could serve as a useful guide to what happens under natural conditions.

E. Evaluation of Techniques Used to Determine Biodegradation Under Biological Treatment Plant Conditions

1. Introduction

Numerous chemicals which potentially could enter the environment first pass through biological waste water treatment plants. Their fate in these systems can be the determining factor in whether the chemicals become environmental pollutants or degrade to innocuous materials. This section will evaluate the techniques used to assess the biodegradability of compounds under sewage treatment conditions.

In general these techniques can be divided into two categories (1) static or screening techniques such as respirometry, river die-away, shake culture, etc. and (2) continuous or semicontinuous techniques. The former techniques have been reviewed in Section III A, p. 49 while the latter were considered in Section III B. p. 126. Static techniques, with the exception of the Warburg method with acclimated seed and sometimes shake culture methods using acclimated seed (e.g. SDA, 1965), provide only limited insight into a chemical's behavior in a waste water treatment plant. Thus this section will concentrate on the continuous and semicontinuous techniques although still considering static techniques when comparisons are made with biodegradability in treatment plants.

For the most part, the compounds that have been studied for their biodegradability in waste water treatment plants have been surfactants (e.g., Swisher, 1970) and the treatment process modelled the most is activated sludge treatment.

As pointed out earlier (p. 126), in evaluating the fate of a chemical, a distinction should be made between "treatability" (removal by physical processes) and "biodegradability". With water-soluble materials the distinction is not much of a problem, since the adsorption sites on the activated sludge will soon become saturated and thereafter the amount of material removed will represent the amount degraded. With water-insoluble materials physical separations may quite often account for all of the removal due to the small amount of sludge that is usually wasted (e.g. Choi et al., 1974).

Important variables which affect the results of the test systems include (1) the chemical structure of the test material, (2) the nature of the biological system, (3) concentrations of the test material, nutrients, and microorganisms, (4) temperature and oxygen and (5) other physical, chemical, or biological factors such as agitation, pH, growth promoters, growth inhibitors, etc. (WPCF, 1967). The impact of some of these factors will be discussed in detail in the following section. The affect of chemical structure is discussed in Section VIII, p. 461 .

2. Factors Affecting Biodegradation Under Waste Water Treatment Conditions

a. Acclimation and Deacclimation of the Microorganisms

The assimilation of an organic compounds by microorganisms may be dependent upon acclimation of the cells to the test compound. Acclimation usually requires varying amounts of time and may involve synthesis of appropriate enzymes, selection of a species, etc. The process is ordinarily a reversible one and, therefore, if the test compound is removed from the feed and then added again, a reacclimation period, usually somewhat shorter, is often necessary. Furthermore, the addition of a readily utilized substance to the feed may result in a repression of the induced enzymes and consequently reduce biodegradation of the test compound (Swisher, 1970; Gaudy _et_ _al_., 1964).

Organic compounds being fed into sewage-treatment plants can be categorized into four not very sharply divided categories (1) readily utilized, (2) utilizable after acclimation, (3) always utilized slowly, and (4) not utilizable. In many cases, surfactants frequently fall into the second category and because they usually are released into sewage in relatively constant concentrations, a major attempt is made to allow maximum acclimation in the test systems used. Other organic chemicals may be released into sewage in varying quantities (shock loading) resulting in great fluctuations in concentration and in acclimation of microbial population. Buzzell _et_ _al_ (1969), aware of the importance of acclimation, purposefully used systems that were not acclimated in order that their results would be representative of slug (shock) loading in activated sludge treatment plants.

Acclimation may be an unpredictable parameter and may change considerably under new conditions. Sludge developed under semicontinuous flow conditions may do poorly for a week or so when transferred to a continuous-flow system. Acclimation may also be dependent on the temperature (see p.202) and source of the seed.

Several approaches have been used in attempts to standardize the seed. The Official German test develops its sludge spontaneously from the growth of bacteria entering from the general environment. Both Buzzell et al. (1969) and Eden et al. (1967) have resorted to the preservation of activated sludge seed. Buzzell et al. (1969) found that lyophilized (freeze-dried) sludge retained the greatest degradation potential. Eden et al. (1967) reported that freeze-drying required the addition of sugar and proteins which might interfere with the biodegradation test so they resorted to using air-dried sludge.

b. Temperature

Although the community of bacteria in activated sludge or trickling filter films may be affected by many parameters such as pH, feed, etc., temperature has been shown in several cases to have a particularly dramatic effect (Ludzack et al., 1961). Using the porous pot technique, Eden et al. (1972) found that while NTA is almost completely removed at 20°C (98 percent), at lower temperatures indicative of winter conditions, removal is considerably less (66-82 percent). Stiff et al. (1973) also studied temperature effects on removal of alcohol ethoxylates and alkyl phenol ethoxylates using the porous pot technique. At 15°, 11°, and 8°C the two alcohol ethoxylate surfactants tested were well degraded. The alkyl phenol ethoxylate was well degraded at 15°C, but at lower temperatures removal depended upon concentration - at 5 mg/l 90% was removed, but at 20 mg/l removal flucturated between 40 to 95% at 11°C and between 20 and 80% at 8°C. Similar poor degradation results were found for alkyl phenol ethoxylates in a field trial during the winter. Stiff and Rootham (1973) studied both linear and branched-chain alkylphenol ethoxylates and concluded that the temperature effect appears to be a feature of these particular materials during treatment, since both types of alkylphenol ehtoxylates exhibited the same temperature effect.

c. Analytical Methods

Variations in analytical methods can provide quite different results for a biodegradation test. An analytical method that monitors disappearance of the parent compound might indicate a high degree of biodegradability, while methods that consider metabolites can give the opposite result if one or more of the metabolites is persistent. For example, Janicke (1971) compared MBAS analysis (see Section III C, 2c, p.154) and total organic carbon (TOC) analysis of LAS in a laboratory activated sludge unit. He found that the TOC method gave a lower rate of degradation thus suggesting that some of the metabolites are not destroyed rapidly.

3. Correlation Between Laboratory and Field Results

Laboratory techniques allow more controlled conditions to be maintained but extrapolation of the results to actual field conditions is not always clear. For this reason several researchers have gone to large scale experiments using full-size sewage-treatment facilities. The approaches used and the inherent difficulties which exist with field tests have been discussed in Section III B.5, p.150.

Detergents have been intensively studied under full-scale sewage-treatment plant conditions. Alkyl benzene sulfonate (ABS) and linear alkylate sulfonate (LAS) have received most of the attention due to the switch over from ABS to the more biodegradable LAS in mid-1965. Brenner (1968) and Weaver (1965) have reviewed a number of the principal field tests. In general, the field results confirm the laboratory conclusions that LAS is more biodegradable than ABS, although the quantitative results vary greatly and seem to be quite dependent upon BOD removal (see Table 17).

Table 17. Summary of Principal Field Test Results
(Brenner, 1968)

Location	Process	Material Used	Detention Time in Hours	% Removal ABS or LAS	% Removal BOD
Manassas, Va.	Conventional Activated Sludge	ABS	6-16	54	89
"	"	LAS	6-16	85	91
Woodbridge, Va.	Extended Aeration	ABS	47	58-61	85-91
"	" "	LAS	47	97.7	94.6
Kettle Moraine, Wis.	" "	ABS	34.5	90.7	96.0
" "	" "	LAS	28.6	96.5	96.0
" "	" "	LAS	34.0	51.3	46.0
" "	" "	ABS	39.0	68.3	84.0
New Lisbon, N.J.	Trickling Filter	LAS	---	75.5	83.0
" "	" "	LAS	---	80.0	---

Mann and Reid (1971a, b) have evaluated alcohol and alkylphenol ethoxylates by field trials with a trickling filter sewage treatment plant which served a small community. These compounds are part of a major class of surface active compounds - the nonionic surfactants. The ethoxylates based on primary alcohols have been found to be readily degradable in laboratory test systems and the field trials confirmed their extensive biodegradability (see Table 18). However, evidence concerning the biodegradability of branched chain alkylphenol ethoxylates is conflicting (Mann and Reid, 1971a). Swisher (1970, p. 248) has attributed this conflict in data to: (1) a failure to make sufficient provisions for bacterial acclimation and (2) a failure of the cobalt thiocyanate (CTAS) analytical method to respond to biodegradation intermediates which still show substantial foaming and other surface activity.

The results of the field trials showed that the alkylphenol ethoxylates tested were biodegradable under summer conditions (15°C - approximately 80% degraded), but were fairly persistent (only 20% degraded) under winter conditions (5°C). The laboratory and field test results of Mann and Reid (1971a) are summarized in Table 18. A TLC analytical technique was used.

Table 18. Comparison of Alcohol and Alkylphenol Ethoxylate Biodegradability under Laboratory and Field Conditions (Mann and Reid, 1971a)

	Percent Biodegraded		
Product	Clear Sewage Effluent Inoculum Die-Away Test	Official German Activated Sludge Test	Trickling Filter Community Trials
Dobanol 25-9[a]	99	95-98 (3 tests)	89
Dobanol 91-9[a]	--	97-98 (2 tests)	83
Nonidet P40[b]	10	20	--
Nonidet - Summer[b]	--	--	ca. 80
Nonidet - Winter[b]	--	--	ca. 20

[a] Alcohol ethoxylate [b] Alkylphenol ethoxylate

Stiff and coworkers (1973) have been able to duplicate this climatic affect on alkylphenol ethoxylate degradation in the laboratory using the porous pot activated sludge technique.

4. General Comparison of Laboratory Methods

a. Biodegradation Potential, Reproducibility, and Directed Comparisons of Techniques

Because of the cost involved and poor reproducibility of the conditions, field test evaluations of chemicals have been only infrequently undertaken. Only thirty such tests have been noted by Swisher (1970, p 190) and for the most part these tests have been almost totally restricted to surfactants. Thus biodegradability information on chemicals under sewage treatment conditions is provided mostly by laboratory and bench scale techniques.

These laboratory scale techniques can vary from extremely simple screening tests, such as the river die-away test, BOD, or Warburg technique, to tests simulating the dynamic process of biological treatment of sewage in a sewage works, such as the official German test, the SDA (1965) semi-continuous activated sludge test, or the miniature activated sludge technique used by Swisher (1970). In general, "the simple screening tests, though giving a rapid indication of the ease with which unacclimatized bacteria will degrade the detergent [or test chemical], will in most cases under-estimate the degree of removal of the detergent during protracted sewage treatment" (Stennett and Eden, 1971) because the screening tests fail to consider adsorption on the activated sludge followed by oxidation or "wasting" of excess sludge.

The laboratory techniques may also vary in the degree of what Swisher (1970) has termed "biodegradation potential." Methods that possess a low potential are generally the ones which use a low concentration of bacteria (e.g., closed bottle test and shake culture test - TBS degradation 5 to 10%). On the other hand, although the river die-away test has a low bacterial concentration, its biodegradation potential, at least for anionic surfactants, is quite high (TBS degraded to the extent of 65-75%) presumably due to the natural species distribution and natural medium (Swisher, 1970). A number of researchers have reported direct comparisons of biodegradation methods for a variety of surfactants that serve to illustrate the different results obtained with different techniques. Truesdale and coworkers (1969) have compared five different test methods with a variety of different surfactants. The results are tabulated in Table 19.

Table 19. Comparison of Biodegradation Test Methods
(Truesdale *et al*., 1969)

			Percent Removed[a]		
Detergent	Pilot Scale Percolating Filters	Standard Aeration Test	Official German Test	SDA Semi-Continuous Sludge	Recycling Trickling Filter
Dobane JNX LAS	92	89-91 (9)	93-94 (2)	96-97 (4)	90-93 (6)
Dobane JNQ LAS	96	95-96 (4)	97-98 (2)	98-99 (4)	96-99 (6)
Dobane 055 LAS	96	96-97 (2)	95-97 (2)	98 (4)	94-98 (10)
ABS-4[b]	90	28-42 (9)	66-68 (3)	98-99 (4)	96-98 (6)
Dobane PT TBS	72	15-22 (3)	34-36 (2)	75-76 (2)	86-91 (5)
Empilan KM9[c]	--	98 (2)	--	99 (2)	98-99 (6)
Empilan KM20[d]	--	98 (2)	--	--	96-99 (6)

[a] Figures in brackets denote number of separate determinations.
[b] "Slow to Acclimatize"
[c] Linear primary alcohol E_9.
[d] Linear primary alcohol E_{20}.

The standard aeration test uses an air-dried activated sludge inoculum (30 mg of dry matter per liter) with standard BOD dilution water and is basically a die-away test. A similar comparison (Table 20) was undertaken by Cook (1968) in her study of the "slant culture" technique. Some general conclusions can be reached from these two studies. The recycle trickling-filter technique is of little use for distinguishing between biodegradable and non-biodegradable compounds because it is so active that it degrades just about everything (even TBS). The SDA semicontinuous method comes closest to duplicating the "true" biodegradability of the compounds if one assumes that results from the pilot

Table 20. Comparison of Biodegradation Test Methods (Cook, 1968)

	Percent Removal, MBAS			
	Dobane JNX	Dobane JNQ	Dobane 055	Difficult to Degrade ABS
Continuous Sludge (Official German)	61 ± 5.2	66 ± 2.9	75 ± 5.0	34 ± 5.5
Slope Culture	74 ± 8.8	89 ± 1.6	0 - 66	20 ± 7.3
River Water	88 ± 0.9	93 ± 0.6	96 ± 0.3	29 ± 1.9
Shake Culture (SDA standard method)	88	96	91	34
Semicontinuous Sludge (SDA, 1965)	89 ± 0.4	96 ± 0.3	98 ± 0.3	70 ± 4.0
Recycle Trickling Filter	92 ± 1.6	96 ± 0.7	97 ± 0.4	83 ± 1.5

scale percolating filter are the "true" value. For other compounds besides the surfactants studied, this similarity in results may not hold true. Cook (1968) concluded that the slant culture technique "gave variable results which were not reproducible and the degradation achieved was very low in comparison with that achieved by other methods."

These two comparison studies also demonstrated the fact that the reproducibility of all the methods decreases for compounds which are relatively non-biodegradable. In both the studies, the range of values is the widest for TBS compounds or difficult-to-degrade ABS materials. Similar low reproducibility is noted for a tetrapropylene derived ABS in a cooperative study organized by the Soap and Detergents Association (SDA, 1965). In contrast, the confidence limits for six LAS samples (very biodegradable) in both the shake flask and semicontinuous test were very close. The reproducibility is also quite dependent upon the analytical technique as has been demonstrated with non-ionic surfactants (SDA, 1969).

b. Advantages and Disadvantages of Individual Techniques

(i) Screening Tests

As noted earlier, screening tests, such as the BOD, river die-away, Warburg, and shake culture tests, provide important biodegradability information on compounds which require little bacterial acclimation. Some acclimation, however, is possible with the Warburg and shake culture technique. The SDA shake culture technique provides for acclimation by two transfers on the medium containing the test chemical and the Warburg seed is often acclimated to the test compound in a semi-continuous activated sludge apparatus (which does not mean the seed is necessarily acclimated under Warburg conditions). Also, the information from these screening tests is quite pertinent to chemicals which periodically enter sewage treatment plants (slug loading). Buzzell *et al*. (1969) have found that a combination of Warburg respirometer and shake culture technique with a standardized seed can provide good indications of slug loading biodegradability.

The BOD test provides little insight into the biodegradability of compounds under treatment plant conditions. The bacterial concentration is not representative of activated sludge conditions and the biodegradable potential is low. Under usual conditions, the test takes 5 days and only the 5-day biodegradation rate is measured. Finally, the oxygen uptake measurement, unless close to 0 or 100% of theoretical, is difficult to relate to test chemical loss or metabolite formation.

The Warburg technique provides incubation conditions more similar to activated sludge conditions and allows for the study of biodegradation rate. However, the short experimental exposure time, although it makes the method faster, allows only minimal acclimation. The oxygen uptake measurement has the same difficulty as mentioned for the BOD test.

The bacterial concentration in the river die-away test is low compared to activated sludge systems, although biodegradation potential, at least for some detergents, is high. The reproducibility from laboratory to laboratory has often been cited as being relatively poor, attributable perhaps to the different biological make-up of various river waters. In order to measure the loss of test chemical some analytical development effort may be necessary. Metabolites can be but are rarely identified.

The shake culture technique is somewhat similar to the river die-away test, except that the conditions are more standardized (e.g., Buzzell _et_ _al_., 1969; Truesdale _et_ _al_., 1969). Acclimation steps are possible and relatively high concentrations of bacteria can be used. Depending upon the analytical procedure used, both loss of the parent compound and formation of metabolites can be determined and the rate of degradation can be assessed.

(ii) Continuous and Semicontinuous Techniques

Modeling of biological sewage treatment systems is more closely approximated in terms of acclimation, agitation, and removal of metabolites with continuous and semicontinuous techniques. Both the bacterial concentration and biodegradation potential are similar and the process of adsorption on the activated sludge can take place in the systems.

The difference between semicontinuous and continuous systems is quite considerable in terms of space, time, and money for installation, operation, and maintenance (Swisher, 1970). The batch unit is much more economical since it does not require the constant attention of a continuous system and uses much less feed and test material (which may be an important factor if the chemical is still in the development stage where only small amounts of the test compound are available). Also, in some continuous techniques, maintaining stable biochemical operation and satisfactory circulation of sludge may be difficult.

Because the official German activated sludge test has been required by German law since 1964, a number of laboratories are equipped to perform the test and a great amount of experience with anionic detergents has been gained. The technique can be run in any well equipped laboratory (no sewage effluent or sludge needed as inoculum) and has been adopted by OECD (Organization of Economic Cooperation and Development) and the Council of European Communities (Council Directive, 1973). Disadvantages include the fact that several researchers have found it difficult to maintain a stable biochemical operation and satisfactory circulation of sludge (e.g., Stennett and Eden, 1971; Truesdale et al., 1969). In some cases, the Germans have added ferric hydroxide to weight the sludge flocs to facilitate rapid settling, which takes the test even further from conditions of commercial operations. In addition, Truesdale et al. (1969) have termed the operation of the system as "laborious." As noted earlier, the reproducibility is less for poorly biodegraded chemicals. Fischer (1965) reports a spread of about ±5%, ±2%, and ±1% for substances with degradation of 75-85%, 85-90%, and 90-95%, respectively. Considerable time may be necessary for acclimation of the system (4 weeks--Houston, 1963) and the test period is 3 weeks. The porous pot modification by Stennet and Eden (1971) seems to remedy the difficult sludge circulation problem.

Miniaturized continuous-flow activated sludge systems provide economy in preparation, storage and handling of feeds as well as savings in time and labor. However, they introduce the possible difficulties of "(1) further departure from characteristics of full scale treatment plants, (2) unsteady biological performance, and (3) limitations on sizes of samples which may be withdrawn for analysis"(Swisher, 1970, p 168).

Swisher (1970) has concluded that these are not serious since (1) the large scale units are so far removed from full scale that another factor of 10 probably has little effect, (2) biological performance is not any more unstable than large scale units and (3) an ample analytical sample is available for surfactant work (MBAS). The last factor might be important for other chemical groups where a less sensitive (than MBAS) analytical technique must be used. The technique of Sweeney and Foote (1964) used natural sewage and fresh seed to shorten the acclimation period. However, as with the river die-away test, the variability of natural feed and seed may result in low reproducibility and perhaps analytical interference. Also, gathering the sewage, sterilizing it, and obtaining the inoculum is time consuming. The turbine agitator may be advantageous when foaming compounds such as detergents are being studied. The miniature unit used by Swisher (1967a, b) is half the size of the unit used by Sweeney and Foote (1964), thus reducing the feed requirements. Both systems are small enough that radiotracer techniques can be readily used (Sweeney and Foote, 1964-^{35}S; Gledhill, 1974-$^{14}CO_2$ evolution).

The semicontinuous operation is advantageous because of the reduced amount of feed and operating attention required. The 24 hour cycle of the SDA (1965) is convenient because it requires no overnight attention. Problems with sludge recycling is also eliminated since a one-half hour settling period is allowed. In addition, the cycling that occurs in a semi-continuous system is similar to many activated sludge processes where the feed and recycled sludge are mixed at the entrance to the aerator.

Trickling filters are somewhat easier to operate than the continuous activated sludge systems, since no sludge needs to be recycled. Also, scale-up factors from laboratory studies to commercial filters can be made without great worry since the most important dimension is the depth of the bed; this is around 6 feet and, therefore, can be easily accommodated in the laboratory (Swisher, 1970, p 156). Furthermore, different degrees of treatment can be studied in a single experiment by taking samples at various depths (Stennett and Eden, 1971). Disadvantages include: (1) a long acclimation period (14 weeks for development of a mature film and 4-8 weeks acclimation - Swisher, 1970), (2) a fly nuisance, (3) lack of easy accommodation in a constant-temperature room or bath, and (4) operational conditions can not be readily adjusted (especially retention time) (Stennett and Eden, 1971). Recirculating filter tests seem to be useless because of their high biodegradability potential.

Anaerobic systems have received little study. However, the general considerations with relationship to die-away and semi-continuous are still applicable. Operation on a semi-continuous basis closely simulates the field conditions in septic tanks and anaerobic digesters and allows acclimation to occur. For compounds that will be treated by septic tanks, an anaerobic biodegradation study may be essential.

F. Cost Analysis

For cost purposes, the techniques used to study the environmental persistence and degradation of chemicals in water systems have been divided into three sections. The first section (1a) consists of preliminary or screening tests which provide information on the relative degradability of materials. The second section (1b) provides costs for intensive studies of metabolic pathways and breakdown product identification. The third section (2) discusses the dynamic techniques used for fairly detailed studies of biodegradation under sewage treatment conditions.

A major cost factor in biodegradation studies is that of the analytical equipment. In prediction of the cost, it has been assumed that an average life of small equipment (e.g., shaker, spectrophotometer, oxygen meter, etc.) is approximately five years (220 working days/year), whereas that of other capital equipment (e.g., scintillation counter, gas chromatograph, etc.) is ten years.

The equipment costs for a test have been estimated on the basis of the number of days required to perform the test, assuming that the instrument will be occupied all that time. The cost for each test is calculated for one compound and for twenty compounds. The labor cost for the test is calculated at the rate of $60/day (MS level worker, including benefits). The overhead is assumed to be 125% of the professional services.

1. Techniques for Studying Biodegradation of Chemicals in Water

a. Preliminary Test to Determine Biodegradability

The cost estimates given in Table 21 are more appropriate for a new laboratory, which is interested in undertaking biodegradation studies. These estimates do not represent the price of the test quoted by a commercial laboratory which is engaged in testing biodegradability on a routine basis.

The results of the preliminary screening test will be helpful in deciding the necessity and approach to be used in more intensive studies. For example, if the preliminary test indicates low biodegradability, the intensive study might use an enrichment culture technique to determine if any breakdown can be detected. If moderate degradation is noted in the preliminary test, the intensive study may concentrate on metabolite identification.

Table 21. Cost Analysis for Preliminary Biodegradability Test

Biodegradation Test Method	Analytical Method	Analytical Equipment needed and their cost	Equipment Cost for the test	Chemicals, glassware and misc. supplies	Labor cost @ $60/day	Overhead 125% of Professional services	Total cost	Cost/compound	Remarks
5 or 10 day BOD (1)	Chemical Analysis	---	---	30	60	75	185	185	
5 or 10 day BOD (20)	"	---	---	300	300	375	975	48	
5 or 10 day BOD (1)	Oxygen Meter	Oxygen meter $400.00	Negligible	40	40	50	130	130	
5 or 10 day BOD (20	Oxygen meter	Oxygen meter $400.00	"	240	120	150	610	30	
Two bottle-single dilution-reaeration method (1)	Chemical analysis	---	---	250 (includes 4 biodegradation units)	120 ($90, if oxygen meter is used)	150	520	520	
Two bottle-single dilution- reaeration method (20)	Chemical Analysis	---	---	2000 (includes 40 biodegradation units)	720 ($360, if oxygen meter is used)	900	3620	181	
Two bottle-single dilution reaeration method (1) of Buzzell et al. (1968)	Carbon analysis Chemical Analysis	Nitrite determination apparatus (colorimeter) $450 High speed centrifuge $1200 Carbon analyser $8000	45	150	600	750	1545	1545	Following parameters are studied in this test: Biochemical oxygen demand, Total carbon, Bacterial population Nitrification
Two bottle-single dilution reaeration Method of Buzzell et al. (1968) (20)	Chemical analysis carbon analysis	Same as above	325	2000	4200.	5250	11775	588	
Warburg respirometry (1)	Warburg Manometer	Warburg Manometer $950 Centrifuge $1200	4	50	120	150	324	324	
Warburg respirometry (20)	Warburg Manometer	"	20	500	600	750	1870	93	
Oxygen electrode respirometer (1)	Polarography	Oxygen polarography $900 Recorder $1000 Temperature control unit $400 centrifuge $1200	7	50	60	75	185	185	
Oxygen electrode respirometry (20	Polarography	Same as above	35	350	360	450	1160	50	
River Die-Away test (1)	Chemical analysis	colorimeter $450	negligible	50	90	110.25	250.25	250	Labor cost could vary from compound to compound depending on the complexity of the chemical method of analysis
River Die-Away test (20)	"	"	"	500	600	750	1850	92	
River Die-Away test (1)	Gas chromatography	Gas chromatograph $8000	8	50	120	150	338	338	
River Die-Away test (20)	"	"	45	700	720	900	2365	118	
River Die-Away test (1)	U.V. Absorption	Spectrophotometer $3000 Centrifuge $1200	4	40	60	75	179	179	
River Die-Away test (20)	"	"	28	300	300	375	975	48	

Table 21. Cost Analysis for Preliminary Biodegradability Test (continued)

Shake culture test (1)	Colorimetry	Shaker $300 Colorimeter $450	Negligible	50	90	110.25	250	250	
Shake culture test (20)	"	Shaker $1500 Colorimeter $450	20	700	600	750	2070	103	
Shake culture test (1)	Gas chromatography	Shaker $300 Gas chromatograph $8000	8	50	120	150	328	328	
Shake culture test (20)	"	Shaker $1500 Gas chromatograph $8000	60	700	720	900	2[illegible]40	119	
Shake culture test (1)	Radioactive material $^{14}CO_2$ measured or parent compound	Scintillation counter, $10,000	10	800 (includes labelled material)	120	150	1090	1090	
Shake culture test (20)	"	" "	115	12000 (includes labelled material)	1500	1875	15[illegible]90	775	
Shake culture test (1)	CO_2 evolution	--	--	75	60	75	210	210	
Shake culture test (20)	"	--	--	700	480	600	1780	89	
Shake culture test (1)	Growth measurement	Spectrophotometer $500	Negligible	50	60	75	185	185	
Shake culture test (20)	"	"	"	450	420	525	1[illegible]95	67	
Shake culture test combined with oxygen utilization and succinic dehydrogenase activity assay (1) (Buzzell *et al.* 1969)	Carbon analysis manometry; succinic dehydrogenase by colorimetric method	Freeze drying apparatus $2500 Warburg respirometer, $950; Centrifuge, $1200; Carbon analyzer $8000; colorimeter, $450	35	100	300	375	810	810	Following parameters are studied in this test: carbon removal oxygen utilization, succinic dehydrogenase activity
Same as above for 20 compounds	"	"	420	700	3600	4500	9220	461	
Model ecosystem (1)	Radioactive material	Scintillation counter, $10,000	36	700 (includes radioactive material and material needed for thin layer chromatography, auto radiography)	480		1816	1816	
Model ecosystem (20)	"	"	375	5000	4920	6150	16445	822	

(The number in parentheses indicates the number of compounds tested)

b. Intensive Biodegradation Study to Identify Metabolites and Elucidate Pathway of Degradation

Once the biodegradability of a compound has been established, a detailed study can be undertaken to elucidate biochemical pathways and characterize the nature of intermediate metabolites formed. For this purpose, pure cultures of microorganisms have generally been considered more suitable. Some researchers have utilized cell-free preparations in these studies; however, the results thus obtained are of very little ecological or environmental relevance. This phase of biodegradation test is time consuming and expensive and, therefore, should be undertaken only with those compounds for which preliminary evidence for accumulation of an intermediate metabolite has been obtained. For example, the CO_2 evolution or BOD is substantially lower than theoretically possible. In the intensive study, radiolabelled material is generally used, which may sometimes have to be custom synthesized. Organic synthesis of a number of suspected metabolites may be needed in order to identify the unknown metabolites of the test chemical. The cost estimate for intensive study of biodegradation is as follows:

One Compound

Labor - Ph.D. level (18K) Six months	9,000
Overhead	11,250
Equipment - GC-Mass Spec. (have analysis performed by a commercial lab) Scintillation counter (cost for six months = $ 500)	2,750
Chemicals, glassware Miscellaneous supplies - material for thin-layer and paper chromatography, autoradiography, etc.	750
Total Cost	$23,500

Twenty Compounds

Labor - Ph.D. level (18K) Three years	54,000
Overhead	67,500
Equipment - GC-Mass Spec. (cost $100,000, depreciate over 10 years; cost for 3 years, $50,000) Scintillation counter, cost for 3 years $3,000.	33,000
Chemicals, glassware and misc. supplies	5,000
Total Cost	$159,500
Cost/Compound	$7,975

2. Techniques Which Simulate Sewage Treatment Plant Conditions

The costs for the major continuous and semi-continuous techniques are detailed in Table 22. The costs of the screening tests have already been reviewed in Table 21. The cost of the combined shake culture-Warburg respirometry screening technique used by Buzzell *et al*. (1969) to study shock loading should be somewhat less than the sum of the individual techniques.

The cost estimates in Table 22 used the same analytical technique so that the difference in the total cost per compound is only a reflection of the procedure. The analytical procedure can drastically affect the total cost, the magnitude of which is indicated previously in Table 21. The costs also demonstrate that continuous techniques are, for the most part, more time consuming and, therefore, expensive.

Table 22. Cost Estimates for Techniques Used to Simulate Sewage Treatment Plant Conditions

Biodegradation Test Method *	Analytical Method	Analytical Equipment Needed and Cost	Test Apparatus Costs	Chemicals, Glassware and Misc. Supplies	Labor Cost @ $60/day	Overhead 125% of Profess. Services	Total Cost	Cost/ Compound
Official German Activated Sludge Test (1)	MBAS	Colorimeter $450	500	500	(7 weeks) 2100	2625	6175	6175
Official German Activated Sludge Test (20)	"	"	2500	5000	8400	10500	26850	1342
Miniaturized continuous activated sludge								
Sweeney & Foote, 1964 (1)	"	"	500	100	(9 days) 540	675	2265	2265
(20)	"	"	2000	2000	3780	4725	12955	647
Swisher, 1967a,b					(30 days)			
(1)	"	"	200	250	1800	2250	4950	4950
(20)	"	"	800	2000	7200	9000	19450	972
Trickling filter (1)	"	"	300	500	(9 weeks) 2700	3375	7325	7325
Trickling filter (20)	"	"	1800	5000	10800	13500	31550	1577
SDA, 1965 semi-continuous activated sludge					(20 days)			
(1)	"	"	100	100	1200	1500	3350	3350
(20)	"	"	1000	1000	4800	6000	13250	662

* The number in parentheses indicates the number of compounds tested.

IV. BIODEGRADATION OF CHEMICALS IN THE SOIL ENVIRONMENT

Industrialization and growth of the human population have resulted in more intensive use of the land for food production and waste disposal. In order to increase food production to meet the need, fertilizers, insecticides, fungicides, nematocides, herbicides and other synthetic chemicals are applied to the land, and the amount of these chemicals used is increasing from year to year. Furthermore, many synthetic organic chemicals such as insecticides are used for public health purposes and they also reach the soil environment. Municipal sewage sludge and waste discharged from industry is ever increasingly deposited on the land either directly or indirectly. Chemicals used as food additives, food preservatives and other household materials are also deposited on the land due to land-filling of municipal wastes.

The chemicals found in soil result either from direct application or indirect application. For the most part, pesticides provide the largest source of direct application. Indirect application results from spray fall-out, from rainfall and dust, from industrial discharge into air and subsequent settling down to soil, from translocation by animal and plant, and from flooding of polluted water on soil. Indirect application can be a major factor in contributing to widespread soil contamination by synthetic chemicals. Only a portion of the chemicals found in soil results from direct application. For example, many soils that have never been treated with DDT contain small amounts of DDT; presumably the chemical comes from spray drift or atmospheric fall-out.

Soil can be viewed as a complex system that consists of chemical reactants and chemical and biochemical catalysts. The biochemical catalysts are composed

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of free-state and absorbed enzymes as well as living cells. Microorganisms seem to be the major contributor to the degradation of these chemicals in soil (see Section II A, p. 33). Chemical reactions and photoreactions (only to a very shallow depth) may also contribute to degradation. This section will evaluate the existing methods used in determining the biodegradation of the synthetic chemicals in soil.

A. Techniques Used for Determining Biodegradation

The existing methods for determining biodegradation in soil can be generally classified into three categories. They include laboratory tests, greenhouse studies and field studies. Field studies reflect the fate of the chemicals in conditions similar to the actual environment. Laboratory tests are carried out under a controlled environment and they are often time saving, convenient and low cost; whereas greenhouse studies are in between laboratory tests and field studies in terms of environmental simulation and complexity. The laboratory tests cover natural communities from soils, pure culture studies, cell-free extract studies and miscellaneous methods.

Quite often researchers have used sterile soil control along with non-sterile soil tests to distinguish between chemical and biological degradation. Controls are also used to distinguish physical removal of the chemical (e.g. due to adsorption) and degradation of the chemical. The commonly used methods of soil sterilization for this purpose are autoclaving, chemical treatment or γ irradiation (see Section V.B.2, p. 352).

1. Laboratory Tests

a. Natural Communities from Soil

The techniques discussed in the following section rely on the use of microorganisms occurring in natural soil. Soils collected from the field are treated with test chemicals in the laboratory. The treated soils may be incubated under (a) aerobic conditions [open air or forced aeration with air] or (b) anaerobic conditions [flushing with nitrogen or other inert gases]. The treated soils may be mixed with water or aqueous solutions containing mineral salts and/or organic substrates and incubated under stationary or perfused conditions, or if an aqueous suspension is used the suspension may be shaken. Methods that involve transferring a portion of the incubated soil to liquid medium containing the test material as well as further transferring are discussed elsewhere (see p. 232).

(i) Soils Incubated with Test Chemicals

This testing method has been widely used. Soil samples are well mixed with the test chemicals and incubated under stationary conditions with or without flushing with air, O_2 (for aerobic conditions), or N_2, $N_2 + CO_2$ (for anaerobic conditions). In this regard, this method is more closely related to the natural systems than the other laboratory testing methods.

Soils collected from the field are generally dried in air and then are passed through a sieve. These soils, in many studies, are subjected to a physicochemical analysis including pH, particle size distribution, percentage of organic matter, ion exchange capacity, total nitrogen content, total carbon content, water-holding capacity, etc.

(a) Aerobic Studies

The majority of the degradation studies are carried out under aerobic conditions. The methods for maintaining aerobic conditions are listed below:

(1) Soil samples after mixing with test material are placed in containers which are open to air. The containers used include test tubes (Castro and Yoshida, 1971), plastic pots (Harris, 1969; Messersmith _et al_., 1971), large jars (Lichtenstein and Schulz, 1959), small rectangular flats (McClure, 1970), and styrofoam cups (Altom and Stritzke, 1973).

(2) Soil samples are placed in containers that are covered but left with air space. The experiments have been performed using differential respirometers (Bartha, 1968, 1969), biometer flasks (Bartha _et al_., 1967; Kazano, _et al_., 1972), buckets and beakers covered with plastic sheets (Bro-Rasmussen _et al_., 1970; Bartha, 1971) sealed or stoppered containers including glass tubes (Hance, 1969; DeRose and Newman, 1947), flasks (Walker and Stojanovic, 1973), jars (MacRae and Alexander, 1965), Warburg flasks (Gilmour _et al_., 1958), covered containers including beakers (Bartha _et al_., 1968), jars with lids or with loose-fitting lids (Zimdahl _et al_., 1970; Montgomery _et al_., 1972).

(3) Treated soils are placed in the containers that are flushed continuously with a low flow rate of CO_2-free air. The containers include wide glass tubes fitted with two-hole stoppers permitting inflow of CO_2-free air and outflow of evolved

CO_2 (Kaufman et al., 1968), pint jars that are connected into an aeration system in an incubator to provide CO_2-free air (McCormick and Hiltbold, 1966), etc.

(b) Flooded Conditions

In order to observe the degradation in flooded soils such as in rice fields or in organic soils below the water table, some degradation experiments are carried out under anaerobic conditions or in submerged soil. For simulating flooded soils, water is generally added to air-dried soils that have been treated with the test chemical previously. Alternatively, aqueous solutions containing the test material are added to the air-dried soil samples. The various ways in which the experiments are set up include:

(1) Air-dried soils are screened to pass through a small sieve, placed in large glass test tubes, and then flooded with water containing test chemical to provide a water level of 5 cm above the soil surface (Castro and Yoshida, 1971; Sethunathan and MacRae, 1969).

(2) Soil samples are treated with the test chemical and are then flooded with distilled water to give a water level of 4.5 cm above the soil surface (Parr et al., 1970).

(3) Soil samples in glass test tubes or glass bottles are flooded with distilled water or with aqueous solution containing test chemical, and the ratio of soil sample and water

(weight basis) is 1:2 (Goswami and Green, 1971), 2:1 (Iwata et al., 1973), 1:1 (Sethunathan and Yoshida, 1969).

(c) Anaerobic Conditions

Strictly anaerobic conditions can be achieved by flushing the soil systems with inert gases such as argon and nitrogen. In most cases, air-dried soils that are screened to pass a small sieve are placed in bottles, and water is added to provide the desired moisture conditions, for example, at 60% field capacity. Incubation is conducted using a multipurpose manifold assembly of the appropriate soil systems with different inert gases or gas mixtures including argon, nitrogen, and $N_2 + CO_2$ (80:20) (Parr and Smith, 1973; Parr et al., 1970; Guenzi and Beard, 1967).

(ii) Soils Suspended in Aqueous Solution

This test method generally uses small amounts of soil samples that are suspended in water containing the test chemicals which may be supplemented with nutrients. The mixture is incubated with or without shaking. There are a few studies in which the soil suspensions containing test chemical are incubated in aerobic conditions. The aqueous solutions contain only mineral salts, and the pH of the solutions is adjusted to neutrality prior to addition of soil samples.

Some researchers have used these conditions with stationary incubation. A small amount of soils serving as microbial inoculum is added to aqueous solution containing the test chemical. The aqueous solutions may contain only mineral salts and test chemical (Miles et al., 1971; Alexander and Lustigman, 1966) or may be supplemented with small amounts of yeast extract, peptone, and glucose to provide an external carbon source (Naik et al., 1972). The aqueous solutions before adding test chemicals are adjusted to a neutral pH. The containers used for the experiments are either flasks or bottles.

(iii) Soil Perfusion Technique

The soil perfusion apparatus has been used for studying detoxification of pesticides and nitrification in soil as well as to study microbial systems utilizing water-insoluble substances such as sulfur. Although each soil perfusion apparatus developed by different people might be different somewhat, the principle they are based upon is very similar. In most cases, the soil percolation apparatus consists of a liquid medium reservoir, a soil column, a delivery tube that transfers solution and air into the soil, an air inlet, and an air outlet (see Figure 24 and 25). The whole unit can be sterile and can be continuously operated under sterile conditions for a long period of time. Most soil perfusion units are operated under negative pressure, although some are operated under positive pressure.

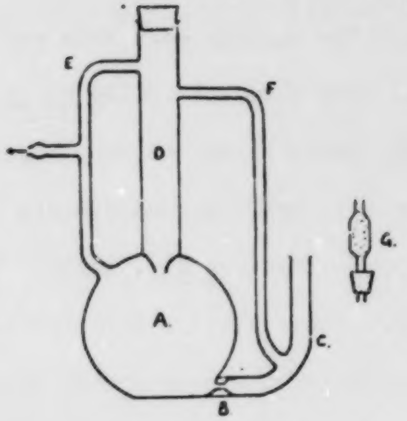

Fig. 24. Soil perfusion apparatus (Collins and Sims, 1956). Courtesy of Nature.

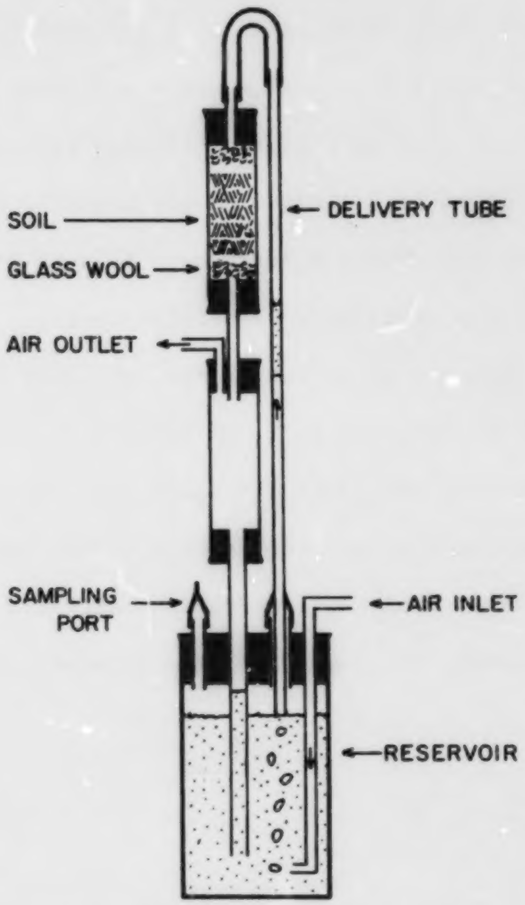
SOIL
GLASS WOOL
DELIVERY TUBE
AIR OUTLET
SAMPLING PORT
AIR INLET
RESERVOIR

Fig. 25. Soil perfusion apparatus (Kaufman, 1966). Courtesy of Weed Science Society of America, publication of Weed Science.

Soils used for perfusion experiments are dried in air and screened through a sieve. Soil samples may be treated with test chemical in the perfusion unit in two ways: (1) soil samples are treated with test chemical before starting perfusions (Kaufman and Kearney, 1965; Kaufman, 1966), or (2) soils are perfused with aqueous solutions containing the test chemical (Audus, 1949; Audus, 1951). The amount of soil employed in a perfusion experiment may vary from 10 to 50 g (air-dried basis), and 200 to 250 ml of distilled water or dilute solution containing a test chemical is often used for perfusion. In most cases, the concentration of test chemical under study is in the range of 5 to 100 ppm. Samples of perfusate can be taken from the reservoir at assigned intervals of time, or daily, for analysis of the chemical residue and the degradation products. The rate of perfusion varies according to the investigator. The method requires either that the parent compound or a product of interest appears in the perfusate.

b. Pure Culture Studies

(i) Pure Cultures Isolated from Soil Enrichments

In order to show soil microorganisms are involved in the degradation of natural and synthetic chemicals in soils, and to establish routes of breakdown it is helpful to isolate microorganisms in pure culture from soils. This demonstrates that a particular microorganism or some combination of microorganisms are indeed degrading a particular chemical or structurally-related chemicals. The most effective and the most commonly employed method for isolating the effective microorganisms is by means of soil enrichment culture techniques. The basic principle behind the techniques consists of increasing the population of the effective microorganisms in the soils or in soil suspensions by treatment with the test chemical. There are three ways of carrying out soil enrichments. These methods are described below:

(a) Enrichment Cultures Obtained by Treatment of Soil with Test Chemical

The technique involves the treatment of soil samples with test chemical solutions. Soil samples may be treated with the test chemical added either to an appropriate mineral salt solution (Burger et al., 1962; Jensen, 1957; Hammond and Alexander, 1972; Tiedje et al., 1973) or to water (Cavett and Woodrow, 1968). In some studies the solution of the test chemical has been supplemented with yeast extract (Belser and Castro, 1971; Ohmori et al., 1973; Jensen, 1957). The treated soil samples are incubated under stationary conditions or on a rotary shaker. After incubation for a period of time, an aliquot of the mixtures is transferred for incubation in a fresh liquid medium containing the test compound. The incubation temperature is generally controlled at room temperature or at 30°C.

The growth of microorganisms can be judged from the increase of turbidity of the enrichment culture fluids, or by microscopic observations. That microorganisms are active in the enrichment cultures can be shown by measuring the decrease in the concentration of the test chemical. For example, this can be easily determined by release of chlorine ion from chlorinated hydrocarbons (Belser and Castro, 1971) or by a decrease in UV absorption of aromatic compounds (Burger *et al*., 1962). For the isolation of an effective microorganism(s) from the enrichment cultures, agar plates of the mineral salt medium containing the same test chemical are streaked with the enrichment culture fluids. The isolates are purified by repeated streaking on the same medium or on a rich medium such as nutrient agar. Each pure culture is then tested for its ability to degrade the appropriate chemical in an appropriate medium. The active microorganism is generally maintained on agar containing the appropriate chemical or occasionally in rich medium such as nutrient agar.

(b) Naturally Enriched Cultures

This soil enrichment technique is similar to the method described above except that natural soil samples which had been treated with the test chemical are used for the source of isolating effective microorganisms without further subcultures. A *Streptomyces* that can degrade herbicide diazinon was isolated from submerged soils treated with the chemical (Sethunathan and

MacRae, 1969). Microorganisms utilizing crude oil have been isolated from soils which had been contaminated with crude oil over a period of time.

(c) Soil Perfusion Cultures

Another soil enrichment technique uses soil perfusion to enrich the effective microorganisms. The technique of soil perfusion has been described previously. Kaufman and Kearney (1965) isolated effective microorganisms on isopropyl-N-3-chlorophenylcarbamate (CIPC) and 2-chloroethyl-N-3-chlorophenylcarbamate (CEPC) from perfused soils. Isolation of pure cultures of effective microorganisms from the perfused soils is accomplished via a soil dilution plate method. Serial dilutions are prepared with the enriched soil from the perfusion units using a mineral salts plating medium. CIPC or CEPC are supplied as a sole source of carbon for organisms obtained from perfused soils treated with these chemicals. The formation of clear zones surrounding certain of the colonies is considered indicative of CIPC or CEPC degradation and utilization. Stock cultures of organisms utilizing either CIPC or CEPC are isolated, purified, and maintained on the mineral salt medium with CIPC or CEPC as the sole source of carbon. Soil perfusion technique has also been used to isolate bacteria that degrade N-methylisonicotinate (Orpin _et_ _al_., 1972) and 4-chloro-2-methylphenoxyacetate (Gaunt and Evans, 1971).

(ii) Other sources of Pure Cultures

Other pure cultures used to study the biodegradation of a compound may be obtained from unenriched soils (Ohmori _et al_., 1973), from sources other than soils (Ohmori _et_ _al_., 1973), and from cultures usually stocked in the laboratory (Miyazaki _et_ _al_., 1970). A method of isolating microorganisms from unenriched soils is carried out by direct sprinkling of soil samples onto an agar medium containing a test chemical (Ohmori _et_ _al_., 1973).

The degradation of synthetic chemicals by pure cultures isolated by enrichment is generally studied in two ways: (1) by growing microbial cells in liquid medium containing the test chemical, and (2) by resting cell techniques. For measuring degradation by cells growing in liquid medium containing the test chemical, generally a small volume of cell suspension is added to a sterile liquid medium containing the test chemical with or without supplemental growth factors such as yeast extract or vitamin B complex. If aerobic conditions are being considered, the mixture may be aerated on a shaker or by bubbling sterile air or sterile CO_2-free air or the mixture may be incubated under stationary conditions. Generally Erlenmeyer flasks are used for this kind of experiment. At assigned intervals of time, a portion of culture fluid is withdrawn for assays of the chemical residue and/or the metabolic products. The incubation time varies considerably depending upon the species of active microorganism, test chemical, type of medium, incubation temperature, aeration, pH of the medium, etc. A species of *Arthrobacter* grown in mineral salt medium containing 0.2% of MCPA as the sole carbon source completely degrades 4-chloro-2-methylphenoxyacetic acid (MCPA) in 100 hours at 25°C under aerobic conditions (Bollag *et al.*, 1967), while a species of *Pseudomonas*, grown in mineral salt medium containing 0.04 M monochloroacetate plus 0.08% peptone completely degrades monochloroacetate in 16 days (Jensen, 1957).

The other way of observing degradation by pure culture is by means of resting cell techniques. Cells grown in the medium containing the test chemical may be harvested in the logarithmic phase of growth (Tiedje *et al.*, 1973), at 18 hours of growth at 30° under shaking condition (Cavett and Woodrow, 1968), after 3-5 days of growth with aeration (Burger *et al.*, 1962; MacRae and Alexander, 1963; Belser and Castro, 1971). They are usually washed with buffer (Cavett and Woodrow, 1968; Belser and Castro, 1971; MacRae and Alexander, 1963). The concentration of phosphate buffer used by MacRae and Alexander (1963) is generally in the range of 0.01M to 0.067M and the pH value between 7.1 to 7.5. Washed cells are then generally suspended in the buffer used for washing the cells, and an appropriate amount of the test chemical is added to the suspension. The mixture in the flask may be stirred slowly at 25°C with a magnetic stirrer

(Belser and Castro, 1971), or the mixture may be incubated in a shaking water bath at 30°C (Tiedje et al., 1973; Sethunathan et al., 1969). Samples are removed periodically for assays of the test chemical residue and intermediate products. If oxygen uptake is to be studied, the mixture is generally placed in Warburg flasks and standard Warburg manometric techniques are followed (Tiedje et al., 1973; MacRae and Alexander, 1963; Cavett and Woodrow, 1968). The dry weight of cells suspended in buffer solution can be obtained by drying similar volumes of the cell suspension and phosphate buffer and the weight difference is the dry weight of cells.

c. Cell-free Extract Studies

For proof that the degradation of the compounds was due to enzymatic reactions, cell-free extracts have been used. In addition, cell-free extracts can also provide information for establishing the pathway of intracellular degradation. The techniques used to promote product accumulation and establish metabolic pathways are similar to those described before (Section III,D,4,b, p. 190).

For preparation of cell-free extract, the cells are often cultured with aeration in a medium containing the test chemical as a source of carbon. The cells are harvested at the late exponential phase of growth (Bollag et al., 1967), or after 2-3 days of growth at 25°C (Orpin et al., 1972), or after 7 days of growth at 27°C (Clark and Wright, 1970) by centrifugation. Before being subjected to disruption, the cells are washed with cold buffer

(Bollag et al., 1967), or buffer supplemented with mercaptoethanol (Orpin et al., 1972). Washed cells are suspended in the same buffer solution and sonicated (Bollag et al., 1967) or disintegrated by high pressure (Clark and Wright, 1970), or by other methods (Heyman and Molof, 1968). If high pressure is used, the cell suspension may be passed through a cooled French press at a pressure of 1,500 psi (Bollag et al., 1967), a Biox X-press or a Hughes' press (Orpin et al., 1972).

Enzymatic activities of the cell-free extract are assayed by incubation of the cell extract in the buffer containing the test chemical. The reaction may be terminated by the inactivation of the enzymes, and the products then are determined. If oxygen uptake and CO_2 evolution are to be determined, conventional respirometric techniques (Warburg apparatus) are employed. Co-factors such as NADH, NADPH or others may be added to the reaction mixture so that enzymatic reaction is enhanced (Buswell and Mahmood, 1972; Orpin et al., 1972).

d. Miscellaneous Methods

Chambers and Kabler (1964) used the Warburg respirometric technique to determine oxygen uptake as an indication of degradation of phenols and phenol-related compounds. The mixed cultures of microorganisms obtained from soil and sediment from a waste lagoon of a catalytic cracking plant were used as the source of inoculum. Pseudomonas species were active in about 80% of the cultures while, in the remainder, Archromobacter, Xanthomonas, and Flavobacterium species were active. Hammond and Alexander (1972) have studied the biodegradability of methyl-substituted aliphatic acids by measuring oxygen utilization by dilution method (for details refer to Section III.A.1.a, p.51) using soil as the source of microbial inoculum.

McClure (1972) reported that a mixed suspension of microorganisms containing at least 7 species of bacteria, fungi and actinomycetes were obtained from an IPC (isopropyl N-phenylcarbamate)-enriched soil sample. The mixed suspension was applied to IPC-treated soil sample to determine whether the suspension can accelerate the degradation of the herbicides in soil as determined by a plant bioassay. Rectangular wooden flats (19 by 23 by 10 cm deep) are filled with composted Cloucester sandy loam soil. Rate of application of IPC equivalent to 4 and 12 kg/ha is homogenized in a nutrient solution. Sixty ml of the mixed suspension (0.5 mg dry weight per ml) is sprayed onto the soil surface of the flat. The flats are placed in the greenhouse and the foliage, if any, is harvested, dried, and weighed after 2 weeks.

Studies of the degradation of alkylbenzene sulfonate (ABS) and herbicides 2,4-dichlorophenoxyacetic (2,4-D), and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in lysimeters have been reported (Robeck et al., 1963). Figure 26 shows the cross section of a lysimeter. The lysimeter contains sandy soil and graded gravel is placed at the bottom of the tank. The experiment is conducted in order to see if the ABS in sewage could be broken down while trickling through an unsaturated soil. To do this, the first 260 days the lysimeter is fed 19 liters of sewage daily at 0.75 liter per minute rate for 25 minutes and then allowed to rest the remainder of the day. During this 260-day period the concentration of ABS in the influent averaged 11.5 mg/ℓ. This concentration of apparent ABS is reduced to 0.5 mg/ℓ in the effluent of the column.

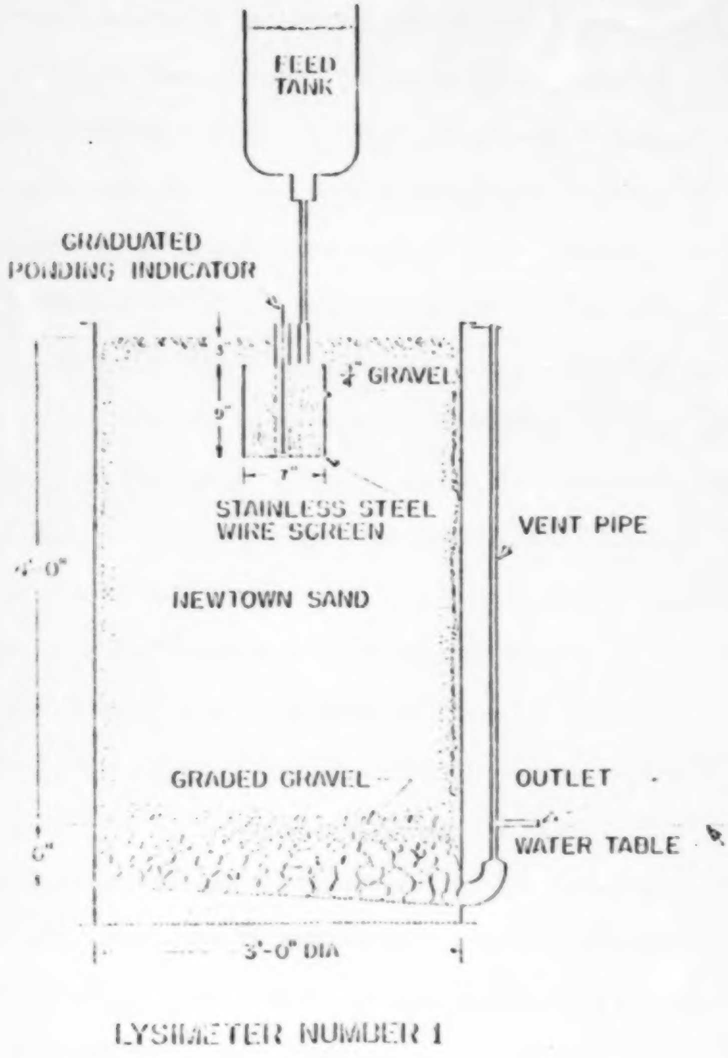
FEED TANK
GRADUATED PONDING INDICATOR
3/4" GRAVEL
9"
7"
STAINLESS STEEL WIRE SCREEN
VENT PIPE
4'-0"
NEWTOWN SAND
GRADED GRAVEL
OUTLET
WATER TABLE
6"
3'-0" DIA
LYSIMETER NUMBER 1

Fig. 26. Cross section of a lysimeter (Robeck et al., 1963). Reprinted with permission from Journal Water Pollution Control Federation, 35, 1225-36, Wash., D.C.

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2. Greenhouse Studies

Only a limited number of researchers have studied biodegradation in soil under greenhouse condition (De Rose and Newman, 1947; Sheets et al., 1968; Burger et al., 1962; Ahmed and Morrison, 1972). In this test, soils obtained from the field are treated with various concentrations of test chemicals before being placed in containers, or conversely the soils are placed in containers then test chemical is applied to the soils. Treated soils are kept in the greenhouse in order to observe the degradation. In most cases the chemicals which have been tested are herbicides including phenoxyalkyl carboxylic acids, benzoic and phenylacetic acids. Determining the toxicity of the treated soils to plants (i.e., bioassay) makes it a simple and convenient way for determining the herbicide residue in soil. Soil treated with herbicide is watered to maintain adequate moisture.

3. Field Studies

Most field studies have been focused on the degradation of organophosphate insecticides, chlorination hydrocarbon insecticides, and chlorinated phenoxyalkyl carboxylic acid herbicides. Some of these compounds, such as DDT and aldrin, can persist in soil for a number of years.

In most studies the field is divided into plots. Each plot receives the test chemical with concentrations generally similar to actual field application. Two kinds of plots in terms of test surface area are employed. One is so-called microplots and the other is regular plots. The surface area of some plots may be smaller than one square foot (Read, 1969; Wolf et al., 1973; Ahmed and Morrison, 1972).

Each microplot is enclosed by a wooden, metal or concrete frame, and may be furnished with a 30 x 30 mesh plastic net bottom. The surface area of regular plots is generally from a few hundred square feet to a few thousand feet (Burnside _et al_., 1971; Lichtenstein and Schulz, 1959; Lichtenstein _et al_., 1970; Schulz and Lichtenstein, 1971; Stewart _et al_., 1971; Stewart and Chisholm, 1971; DeRose and Newman, 1947). Plots are separated individually by ridges of soil or by cultivated strips that are built up around each plot to prevent lateral surface movement of the test chemicals.

The treatment of test chemicals in soil plots is generally carried out by applying the test chemicals evenly over the soil. The chemicals may then be incorporated thoroughly into the soil to a desired depth. In the case of microplots, test chemicals are uniformly mixed by hand with a rake. In most cases, test chemicals are mixed with soil to a depth up to 6 inches, but chemicals applied to a 15-inch depth have been reported (Nash and Woolson, 1967). The amount of test chemical applied to the soils is generally in the range of 2 to 20 lbs. per acre, which is not far from the actual dosage of many pesticides applied to crop fields. Higher concentrations are also used in some studies (Wolfe _et al_., 1973). In many cases, several different dosages may be applied to the soils to compare the persistence of the test chemical in various concentrations. Such field experiments require adequate replication. Furthermore, untreated control plots are often essential. The chemicals can be applied to the soils only once or they may be applied annually for a few consecutive years (Lichtenstein _et al_., 1970; Stewart _et al_., 1971; Stewart and Chisholm, 1971). Soil samples are collected by means of a soil sampler (Ahmed and Morrison, 1972), or be a soil auger (Lichtenstein and Schulz, 1959; Schultz and Lichtenstein, 1971). If the vertical distribution of the chemical residues in the soil plots is to be studied, samples are collected from different depths. Soil samples are then subjected to chemical analyses and/or biological assay.

B. Analytical Procedures

The accuracy of the experimental results from soil degradation studies depends to a great extent upon the analytical methods employed. Good experimental setup without supporting, suitable analytical procedures can make the results meaningless. This is especially true in the case of degradation experiments of synthetic chemicals in soil where the concentration of the test chemicals applied to soils is generally very low and the soil is very complex.

The analytical procedures used for determination of the chemical residues and their metabolic intermediates in soils can be generally divided into two categories: (1) chemical analyses and (2) bioassays. Chemical analyses cover chromatographic methods, spectrophotometric techniques, radioassays, CO_2 evolution, O_2 consumption, and GC-mass spectroscopic techniques. Bioassays include plant assays for herbicides, insect assays for insecticides, etc.

1. Chemical Analyses

a. Extraction and Clean Up Procedures

Prior to analyses, chemical residues and their metabolites in soils have to be extracted from the soil samples and then concentrated.

Soil contains a variety of inanimate and colloidal materials which can react with both the parent and intermediary compound and thereby make their extraction extremely difficult. Divising a technique for extraction of an intermediate may be particularly difficult because the sorption and solubility characteristics of the intermediate are unknown.

Most chemicals under study as well as most of their metabolites are organic in nature, and, therefore, organic solvents are commonly used for extraction. The choice of organic solvent depends on the chemical nature of the test compounds and their metabolites. Very often the solvent may consist of a mixture of two or more solvents and the system chosen is the one that gives the highest recovery yield. For example, acetone is used for removing anilide (Bartha, 1968), organophosphorous insecticides (Bro-Rasmussen, et al., 1970), and dichlobenil (Montgomery, et al., 1972) residues from soils. Hexane and acetone mixtures (1:1) have been used to extract trifluralin (Parr and Smith, 1973), and Dyfonate (Schulz and Lichtenstein, 1971). Sethunathan and Yoshida (1969) used hexane, then acetone and then hexane for diazinon. Extraction can take place by hand shaking, in a mechanical shaker (Iwata, et al., 1973), in a Waring blender (Bartha, 1968), by sonication (Parr and Smith, 1973), or in a Soxhlet extractor (Bro-Rasmussen, et al., 1970; Montgomery, et al., 1972; Stewart and Chisholm, 1971; and Zimdahl, et al., 1970). The extract may be concentrated to an appropriate volume and then directly used for chromatographic analyses (Bartha, 1968; Stewart and Chisholm, 1971). In other cases, the extract needs further purification and/or clean-up in order to avoid unnecessary interference for chromatographic analyses.

One method of clean-up requires that the concentrated extracts be purified by passing though an alumina column (Montgomery, et al., 1972; Bro-Rasmussen, et al., 1970; Lichtenstein and Schulz, 1959). Another method is to add activated carbon to the extract (Bro-Rasmussen, et al., 1970).

The problems involved in separation of the chemical residues and their intermediates in the culture solutions of the pure culture studies or mixed culture studies are generally less complicated and simpler. Unlike soil degradation studies, the chemical residues in the liquid medium of the pure culture studies are generally not associated with soil particles and are, therefore, easier to extract. By and large, the main techniques for separation of the chemical residues and their intermediates from the culture fluids are about the same as the spearation procedures used with soil samples. In that case, the culture solution is extracted with solvent, and the extracts are dried with anhydrous sodium sulfate and then concentrated (Bollag et al., 1967; Miles, et al., 1969). Clean-up may be required for some extracts (Schulz, et al., 1970; Miyazaki, et al., 1970). Furthermore, the culture solutions before being subjected to solvent extraction may have the hydrogen ion concentration adjusted to the appropriate pH by adding NaOH or HCl so that extraction efficiency is enhanced (Bollag, et al., 1967; MacRae and Alexander, 1963).

In most cases, the intermediates in both the culture solutions or soil samples are by no means completely separated by solvent extractions. This is particularly true of many water soluble intermediates.

b. Chromatographic Methods

Chromatographic methods have been widely used for separation and determination of chemical residues and their metabolic intermediates in soil. Among them, gas-liquid chromatography and thin-layer chromatography are most extensively used.

(i) Gas-Liquid Chromatography

When the chemical under study and some of its metabolic intermediates are volatile and thermally stable, gas-liquid chromatography (GLC) provides a good tool for quantitative determination. In most cases, a small volume (a few microliters) of the extract is injected into the instrument. Electron capture and flame ionization detectors are the most commonly employed. Some of the chemicals or their metabolites (e.g. organic acids) that are relatively non-volatile can be converted to volatile derivatives by simple procedures such as esterification. For example, fatty acids (MacRae and Alexander, 1963), MCPA (4-chloro-2-methyl-phenoxyacetic acid)(Bollag, *et al.*, 1967), chloramben (3-amino-2,5-dichlorobenzoic acid) (Wildung, *et al.*, 1968), fenac (Harris, *et al.*, 1969), are methylated prior to gas chromatographic analysis.

(ii) Thin-layer Chromatography

Many organic chemicals and their metabolites can be detected and separated by means of thin-layer chromatographic methods, if the right solid absorbent and the right liquid solvent systems are chosen. By combination with radioisotopic techniques (Sethunathan and Yoshida, 1969; Sethunathan, et al., 1969; Jones and Hodges, 1974; Miyazaki, et al., 1969; Baude, et al., 1974), with GLC (Sethunathan, et al., 1969), or with spectrophotometric methods, quantitative determinations can be achieved. In essence, a small volume of the concentrated extract is spotted to a TLC plate and is developed along with standards with a suitable solvent system in a TLC tank. Sometimes, two or more solvent systems are used for developing the same sample along with standards to ensure the identification of unknowns (Guenzi and Beard, 1967). The TLC plates may contain fluorescent indicators that help to locate the spots under a short wavelength UV light. In most cases, one dimensional TLC is used, but two dimensional TLC may be employed (Schulz, et al., 1970).

(iii) Paper and Column Chromatography

Because of time involved and some limitations in quantitative determinations, paper and column chromatography are not frequently used for separation and determination of the chemical residues and their metabolites. However, column chromatography has been used for fractionating metabolized oil samples for subsequent GLC analyses (Jobson, et al., 1972). Also, a modified ion-exchange resin column technique has been used for paraquat (a herbicide) residues in culture broth (Anderson and Drew, 1972). In addition, a descending paper chromatographic technique has been used for determining metabolic products of

MCPA (4-chloro-2-methylphenoxyacetate) by a soil Pseudomonas sp. (Gaunt and Evans, 1971).

c. Spectrophotometric Methods

Spectrophotometric determination of synthetic chemical residues and their degradation products in soils usually does not have the sensitivity of GLC and TLC analyses. It may not be able to distinguish between the parent chemical, metabolites and hydrolysis products. Nevertheless, it can be used for determining some chemical residues or their metabolites in soils or can be used as a confirmatory technique with TLC or GLC. There are three types of spectrophotometry often used in soil studies: UV (200 - 400 nm), visible (400 - 700) and infrared (2 - 15 μ).

(i) UV Absorption

UV methods for quantitative determinations usually require a rigorous clean-up of the extract to ensure the final solution is free of any interference material in the spectrum region to be measured. However, UV absorptions have been used as an indicator of the decomposition of aromatic compounds including phenoxyalkyl carboxylic acid herbicides, substituted phenols and mono- and dis-substituted benzenes by soil microbial communities or by pure cultures in aqueous solutions (Alexander and Aleem, 1961; Alexander and Lustigman, 1966; MacRae and Alexander, 1963; Burger, *et al*., 1962). Since the analytical procedure involves no extraction and clean-up, soils used for this type of study should release a minimum amount of material that would interfere with UV measurements. The problem can be partially overcome by using very dilute suspension of soil. Alexander and Lustigman (1966) for example, used 45 ml of mineral salts media inoculated with 1.0 ml of a 1% suspension of Niagara silt loam. The authors note that a major shortcoming, imposed by the UV analysis, is the possible unsuitability of the test conditions.

(ii) Visible Spectrophotometry

Since most synthetic chemicals applied to soils and their metabolites are not colored, a colored complex must form before the chemical can be quantitatively determined by a spectrophotometer in the visible region. Colorimetric methods can be used with other analytical methods for confirming degradation. For example, degradation of chlorinated phenoxyalkylcarboxylic acids, such as 2,4-D (2,4-dichlorophenoxyacetatic acid) and MCPA (4-chloro-2-methylphenoxyacetic acid) are shown by a combination of the release of chloride ion (the colorimetric determination), with the loss of UV absorption, loss of radioactivity of parent compound, GC determination, and plant bioassays (Bollag, et al., 1967; Burger, et al., 1962). Kaufman and Kearney (1965) determined degradation of herbicides CIPC (isopropyl-N-3-chlorophenyl carbamate) by two colorimetric methods; one determines the release of chloride and the other, the formation of aniline.

(iii) Infrared Spectrophotometry

Infrared spectrophotometry is mainly used for qualitative determination of degradation products (Miyazaki, et al., 1970; Bartha, 1969; Buswell and Mahmood, 1972). This requires the isolation of a relatively large amount (μg-mg) of pure compound.

d. Radioassays

Radioactive tracer techniques have been a most useful tool for determining intermediary metabolism and detoxication mechanisms (Casida, 1969). The studies of the breakdown pathways of the chemicals (mainly pesticides) in soils are no exception. Radioassay usually can be achieved at nanogram levels of the labelled compound, and the specificity of analysis depends largely on the degree of separation and clean-up before scintillation counting or radioautography. Chemicals in soil studies are generally labelled with ^{14}C because of the required long test period and because the detection of $^{14}CO_2$ evolution is a good indication of mineralization. The selection of the site for labelling is very important and depends upon what information one wants to obtain. For example, if one is interested in the cleavage of the acetate moiety from the aromatic ring of 2,4-D (2,4-dichlorophenoxyacetate) and in the fate of acetate moiety in soil, the acetate moiety should be labelled with ^{14}C. On the other hand, if one is interested in the degradation pathway of the aromatic rings, the rings will be labelled with ^{14}C (Tiedje et al., 1969). The use of ^{14}C-labelled chemicals in soil studies has been divided into three sections: (i) Assay for the loss of radioactivity of the test chemical; (ii) Identification of metabolic intermediates; (iii) $^{14}CO_2$ evolution.

(i) Assay for the Loss of Radioactivity of Test Chemicals

The accuracy for the determination of the loss of radioactivities of test chemicals from soil samples largely depends on the extraction and clean-up. These procedures have been previously discussed (see p. 243). To separate the parent ^{14}C test chemical from its metabolites, TLC is usually employed. Then the spots are scraped and placed in a scintillation fluid for counting radioactivity (Jones and Hodges, 1974; Bollag and Liu, 1971) or the radioactivity may be determined by a radioscanner (Baude, et al., 1974). The determination of total radioactivity remaining in the soil samples (residual ^{14}C-labelled parent compound plus any nonvolatile ^{14}C-labelled degradation products) can be done by combustion techniques (Baude, et al., 1974; Kazano, et al., 1972). In the case of pure culture studies, the total radioactivity in culture fluid is determined by directly transferring a portion of culture fluid to a scintillation fluid and counting (Bollag, et al., 1967; Bollag and Liu, 1971). Radioassay techniques are also applied in studies of the leaching of chemicals in soils and binding to soil components (Jones and Hodges, 1974; Kazano et al., 1972).

(ii) Identification of Metabolic Intermediates

Again the accuracy of results relies on the extraction and clean-up. The ^{14}C-labelled products and the ^{14}C-labelled parent compound are generally separated by TLC. Standards of the probable metabolites of the test chemical (when they are known) are cochromatographed with the unknown (Goswami and Green, 1971; Sethunathan and Yoshida, 1969; Miyazaki, et al., 1970; Schulz, et al., 1970; Orpin, et al., 1972; Matsumura, et al., 1971). The radioactive spots in plates are located by means of radioautographic technique or by a radioscanner. By comparison of the R_f values of the unknown metabolic products with the standards, the possible identity of the unknown metabolites may be obtained. With the assistance of radioautography or radioscanner, the portions of unknown ^{14}C-labelled metabolites are detected, each portion is collected, extracted and crystallized. When large enough amounts of the unknown are available, the unknown can be identified with analysis by IR, NMR, mass spectroscopy, etc. Besides TLC, GC has been used for separating ^{14}C-propanol (a degradation product of 1,2-dibromo-3-chloropropane) from soil samples and the n-propanol is trapped from GC by bubbling through a dioxane scintillation solution (Castro and Besler, 1968).

(iii) $^{14}CO_2$ Evolution

Although the disappearance of the radioactivity of ^{14}C-labelled test chemicals in soil samples is a good indication of degradation, the detection of $^{14}CO_2$ evolution provides a definite indication of the mineralization of the chemical, since water and CO_2 are the final biodegradation products. Soil samples treated with a ^{14}C-labelled test chemical (may be also treated with the same non-labelled chemical) are generally placed in a glass container and incubated under aerobic conditions (Kaufman, et al., 1968; Jagnow and Haider, 1972; Kazano, et al., 1972; Wildung, et al., 1968; MacRae and Alexander, 1965). For the studies of $^{14}CO_2$ evolution under submerged soils, evolved $^{14}CO_2$ is periodically withdrawn by passing CO_2-free humidified air (Goswami and Green, 1971). Any evolved CO_2 is adsorbed in a gas trapping solution. The CO_2-trapping solution is either a mixture of alcohol and amine (for examples, ethanolamine and 2-methoxyethanol, 1:2; ethanol and ethanolamine, 2:1, etc); or KOH or NaOH solution (0.1 N or 1N). The radioactivity of $^{14}CO_2$ in the trapping solution is determined by transferring a known volume of the trapping solution to a scintillation solution and counting in a scintillation counter (Miyazaki, et al., 1969; Skipper and Volk, 1972; Kazano, et al., 1972). If a KOH or NaOH solution is used for trapping CO_2, the radioactivity of $^{14}CO_2$ can also be determined by converting it to $BaCO_3$, and its radioactivity is then counted (MacRae and Alexander, 1965).

e. GC-MS Techniques

Although the gas chromatograph is an effective instrument for the separation and quantification of degradation products, it provides little insight into the identity of the chemical structure of the residue. For that reason, several researchers have resorted to using gas chromatrography combined with mass spectrometry. Both Hammond and Alexander (1972) and Tiedje et al. (1973) have used the technique with extracts from pure culture studies. The extraction procedures and derivatization techniques are the same as used with gas chromatography alone. Detection and identification of amounts as low as 10^{-11} grams are possible (Karasek and Laub, 1974).

f. O_2 Consumption

Although respirometric techniques have not been applied as frequently in recent years in studies of biodegradation in soil as they have in water, they sometimes are used. In a study of the biodegradation of various aliphatic acids, Hammond and Alexander (1972) used standard BOD dilution bottles containing a nutrient salts media and enough organic acid to utilize 8.3 mg/l of dissolved oxygen (inoculum 30 mg of soil). The oxygen uptake was measured with an oxygen electrode. Oxygen utilization resulting from nitrification and oxidation of soil organic matter was corrected for by subtraction of an untreated (no organic acid) blank.

A number of researchers have isolated pure cultures from soil and then measured O_2 consumption of the cells suspended in nutrient medium with the test compound (Orpin *et al.*, 1972; Burger *et al.*, 1962; Fincher and Payne, 1962; Tiedje *et al.*, 1973; Clark and Wright, 1970). Warburg respirometry is most commonly used. Similar procedures have been used with cell-free extracts (Neujahr and Varga, 1970; Buswell and Mahmood, 1972).

Many studies have used respirometry techniques with natural soil samples. For example, Gilmour and coworkers (1958) used 125 ml Warburg flasks to study the oxidation of glucose and wheat straw in soil samples. In a study of the biodegradation of anilide herbicides in soil, Bartha (1968) used a constant-pressure differential soil respirometer constructed in his laboratory to measure O_2 uptake. The apparatus consisted of a 250 ml filter flask connected to individual manometers. Glass syringes, connected to the apparatus by a syringe needle, are adjusted to the uppermost mark initially. The O_2 uptake is measured by returning the manometer fluid to its original position by adjustment of the syringe. The results were reported as the difference between treated and control soil samples and in all cases negative values (representing inhibition) were noted at the chemical concentrations employed.

g. CO_2 Evolution

As was mentioned under Section d., Radioassays, p. 250, the measurement of $^{14}CO_2$ evolution from soil treated with a ^{14}C-radiolabelled material is a good indication of the mineralization of the test chemical. However, several researchers have used unlabelled CO_2 evolution as an indication of the rate of respiration of the added test compound (Parr and Smith, 1973; Messersmith *et al.*, 1971; Engvild and Jensen, 1969). Messersmith and coworkers (1971) measured the CO_2 evolved from a positive pressure soil perfusion system described by Kaufman (1966) (see Section on soil perfusion technique, p.229). DeFrenne *et al.*, (1973) measured the CO_2 evolved from cell-free extracts used to metabolize 2-hydroxy-muconic acid. The technique is basically the same as described for $^{14}CO_2$ evolution (trap CO_2 with base), except that a control must be run to subtract the endogenous respiration.

2. Bioassays

The determination of the soil persistence of pesticides, including insecticides and herbicides, often uses bioassays. This is especially true with field tests. The method can show the persistence of a pesticide residue toxic to sensitive animals or plants in soil. Furthermore, bioassays are desirable because they do not rely on extraction techniques and are relatively inexpensive because they do not require the development of an analytical procedure. A disadvantage of the method is that pesticides may form complexes with soil or firmly bind to soil and become non-toxic to sensitive animals or plants, even though the same concentration in aqueous solution would be very toxic.

a. Plant bioassays for herbicides

The bioassays for the determination of the persistence of herbicides in soils are based on the growth inhibition of sensitive plants. The degree of growth inhibition is generally correlated with the concentration of herbicide in soil as long as the concentration of the herbicide is not too high or low. The parameters used for determination of a herbicide in soil are total dry weight of the plant (McClure, 1972), total dry weight of the aerial portion (Lavy, et al., 1973; Messersmith et al., 1971), dry weight of seed yield per acre (Burnside et al., 1971), fresh weight of shoots (Sheets, et al., 1968), lengths of roots (MacRae and Alexander, 1965), and percentage of emergency and normal development (MacRae and Alexander, 1965). Table 23 lists a number of plant bioassays used to study herbicide persistence.

For field bioassays, soil plots receive various concentrations of herbicide. Seeds are then planted at various intervals. For laboratory and greenhouse bioassays, soil samples to be tested are generally placed in pots, treated with herbicide, and then the seeds are planted.

Table 23. Plant Bioassays for Herbicides

Reference	Compounds Studied	Plants	Time of Planting	Remarks
DeRose and Newman (1947)	dicamba picloram 2,4-D ;2,4,5-T	Soybeans and oats	1st day of herbicide treatment and subsequent plantings	Field bioassay
Burnside et al., (1971)	dicamba picloram 2,3,6-TBA	Field beans Amsoy beans	Two years after treatment, every year thereafter	Field bioassay
Messersmith et al., (1971)	trifluralin	Corn	Right after herbicide treatment	Greenhouse bioassay
Sheets et al., (1968)	2,3,6-TBA dicamba tricamba	Snap beans	Right after herbicide treatment	Greenhouse bioassay
Lavy et al., (1973)	2,4-D	Soybean	Right after herbicide treatment	Greenhouse bioassay
Audus (1949, 1951)	2,4-D MCPA 2,4,5-T	Cress roots	—	Perfusate from soil perfusion column

b. Insect Bioassays for Insecticides

The persistence of insecticides, such as organophosphates and DDT, can be determined by bioassays using sensitive insects. The technique has been used by Ahmed and Morrison (1972), Harris (1969), and Read (1969). In most cases, insect larvae are placed in the soil sample and the analysis is determined by the number that survive.

C. Evaluation of Biological Techniques

1. Factors Affecting Degradation

The key factors that affect the degradation of synthetic chemicals in soil appear to be soil type, depth of soil, test chemical concentration, soil microorganisms and acclimation, physical environment (including pH, temperature, O_2 availablility, redox potential and moisture content of the soil), and external carbon source.

Studies which deal with the influence of these factors on degradation of chemical compounds in soil are described below. However, a large number of studies in the literature have determined persistence of a chemical as a function of changes in the environmental conditions, but have not distinguished between degradation and loss due to movement, adsorption, volatilization, and minor alteration in the molecule. Keeping in view the scope of this report, such studies have not been cited as examples in this section.

a. Soil Type

A very wide spectrum of soil types has been used in studies of the degradation of synthetic chemicals in the soil environment. The soil types vary from sand (low in organic matter content, usually less than 1%) to muck soil or peaty soil (high in organic matter content, usually higher than 50%). Although it is known that soil type does influence the degradation of synthetic chemicals in soil, it is less certain whether the rate of degradation correlates with organic matter content of the soil. Considering the fact that microbial activity is associated with organic matter content, the soil types that have higher organic matter content should give higher degradation rates.

However, the presence of organic matter in soil also affects the movement, adsorption and vaporization of the chemical (e.g., see Broadbent, 1967) and results in immobilization of free-state enzymes. Such effects may reduce the chances of cells or enzymes finding their substrates and thus perhaps reduce the degradation rates. In fact, Marshall (1971) has shown a decrease in the level of metabolic activity due to adsorption of cells and enzymes on soil organic matter.

Kazano *et al*. (1972) found the rate of degradation of carbaryl (1-naphthyl methylcarbamate) was influenced by soil type; $^{14}CO_2$ evolution in six soil types varied from 3 to 35.2% of initial radioactivity during 32 days of incubation (Table 24).

Table 24. $^{14}CO_2$ Evolution from Five Soil Types Each Receiving 2 ppm of ^{14}C-Carbaryl (Incubation period: 32 days) (Kazano *et al*., 1972)

Soil Type	Organic Matter (%)	pH	$^{14}CO_2$ Evolved (%)
Clay	5.8	5.2	7.6
Sandy loam	3.3	5.3	33.9
Clay loam	12.8	6.1	35.2
Loamy sand	1.5	6.3	3.0
Loam	4.1	5.6	5.0

Kaufman et al. (1968) studied the degradation of amitrole (3-amino-1,2,4-triazole) in many soil types and found that the most rapid and extensive degradation of amitrole occurred in the silty clay loam soil (Figure 27).

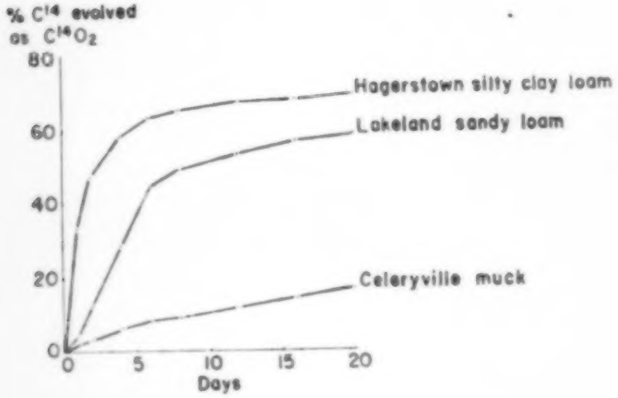


Figure 27. Effect of Soil Type on Amitrole Degradation (Kaufman et al., 1968)
Courtesy of Weed Science Society of America, publisher of Weed Science.

b. Soil Depth

With an increase of soil depth, there is generally a decrease in oxygen tension, volatilization, and loss of the test chemical. A change in the types and abundance of microorganisms also accompanies a change in soil depth. Some characteristics of two soil types (Sharpsburg silty clay loam and Keith sandy loam) at three depths are shown in Table 25. Lavy et al. (1973) studied the degradation of 2,4-D and atrazine in

Sharpsburg silty clay loam and Keith sandy loam at various depths. They found that whereas the degradation of 2,4-D was rapid in soil from all the depths tested, the rates of degradation of atrazine decreased with an increase in soil depth of the two soil types.

Table 25. Characteristics of Sharpsburg Silt Clay Loam and Keith Sandy Loam at Various Depths (Lavy _et al._, 1973)

				Percentage		
Soil Type	Soil Depth (centimeters)	pH	Count of Microorganisms x 10^5/g	Organic Matter	Clay	Sand
Sharpsburg Silty Clay Loam	0 - 23	6.2	26	4.8	39	15
	36 - 61	6.1	16	1.3	42	25
	91 - 122	6.7	3	0.8	38	25
Keith Sandy Loam	0 - 23	7.5	18	0.9	18	77
	36 - 61	8.1	3	0.6	21	39
	91 - 122	8.2	3	0.1	17	35

c. Test Chemical Concentration

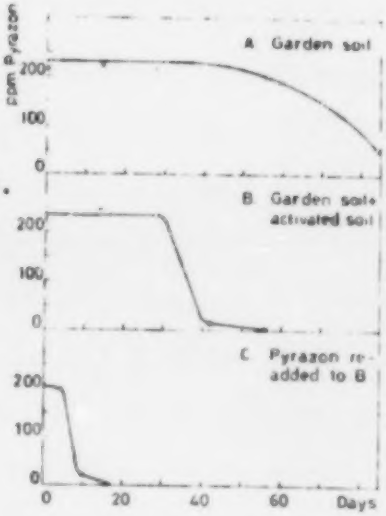
The persistence of synthetic chemicals in soil is often affected by the dose applied to soil. At higher concentration, the chemical often persists longer than at low concentration. Ahmed and Morrison (1972) found that the longevity of four organophosphate insecticides was always longer at a higher rate of application to soils. Similar observations were made by Wolfe et al. (1973) with the organophosphate insecticide, parathion. Messersmith et al. (1971) studied the rates of breakdown of the herbicide trifluralin in two soil types treated at the concentration of 140 and 1 ppm. The rate of $^{14}CO_2$ evolution from ^{14}C-trifluralin was slower at 140 ppm of the herbicide than at 1 ppm.

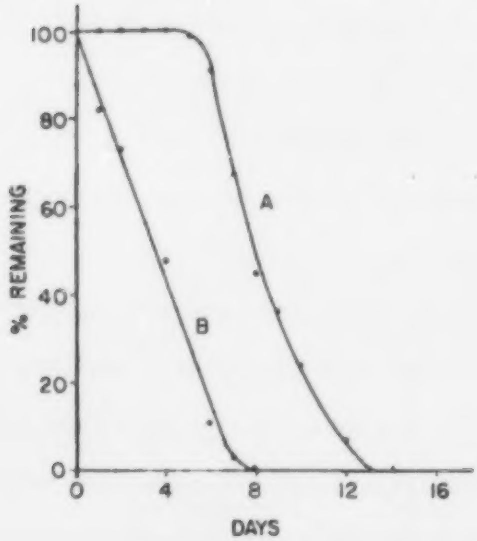
d. Soil Microorganisms and Acclimation

Organic chemicals in soil are broken down largely by microorganisms. Soils differ in the types and abundance of microorganisms they contain and this affects the persistence of chemicals in soil. Degradation of organic chemicals by different soil types has been discussed previously (p.259). Although degradation rates vary considerably from one soil type to another, the variations cannot be attributed exclusively to the microbiological composition of the soil, since in complex environments such as soil, other soil and climatic factors such as temperature, moisture content, acidity, organic matter, etc. are also critical factors which affect degradation.

Acclimation also affects the capacity of soil microbes to inactivate chemical compounds. Evidence for acclimation of soil microorganisms to a test chemical has been obtained by a number of researchers. Engvild and Jensen (1969) found that in the previously untreated garden soil, the breakdown of herbicide pyrazon was not complete even after 3 months. Addition of 10% pyrazon-incubated soil initiated a rapid breakdown of the herbicide and degradation was complete within 45 days (see Figure 28). Kaufman and Kearney (1965) reported that phenylcarbamate herbicides (isopropyl-N-3-chlorophenylcarbamate [CEPC]) were degraded more rapidly when reapplied to the enriched soil and perfused for the second time, as is demonstrated in Figure 29 and 30.

Figure 28. Time Course of Breakdown of 250 ppm of Pyrazon in Different Soils
A. Untreated garden soil (No. 3).
B. Addition of 10% pyrazon decomposing soil (No. 1) to untreated garden soil (No. 3) causes accelerated breakdown.
C. The garden soil has become enriched with pyrazon decomposers and further additions of pyrazon are rapidly decomposed.
(Engvild and Jensen, 1969)
Courtesy of Pergamon Press Ltd.





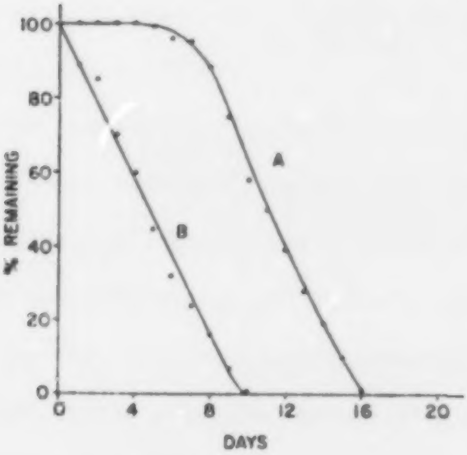


Figure 29. Disappearance of CIPC in (A) perfused soil treated with CIPC, and (B) same perfused soil after a second treatment with CIPC.
(Kaufman & Kearney, 1965)
Courtesy of American Society for Microbiology

Figure 30. Disappearance of CEPC in (A) perfused soil treated with CEPC, and (B) same perfused soil after a second treatment with CEPC.
(Kaufman & Kearney, 1965)
Courtesy of American Society for Microbiology

e. Physical Environment - pH, Temperature, Oxygen Availability, Redox Potential and Moisture Content of the Soil

The hydrogen ion concentration of the soil or of the degradation medium can affect the rate at which both chemical and microbiological decomposition occur. For biodegradation, the pH effect is generally very specific, depending on the particular chemical and the particular microbe involved. The herbicide dalapon was detoxified in soil much more rapidly at pH 7 than at pH 4, whereas 2,4-D was detoxified more rapidly at pH 5 than at pH 7.0 (Corbin & Upchurch, 1967). Organophosphate insecticides have been reported to persist longer in acid soil (Griffiths, 1966). However, Konrad *et al*. (1967) and Getzin (1968) found that the organophosphate insecticide diazinon decomposed much faster in acid soils. With chlorinated hydrocarbons, researchers have generally found no correlation between their persistence and the pH of the soil (Swanson *et al*., 1954; Fleming and Maines, 1953; Bollon *et al*., 1958). Corbin and Upchurch (1967) have reported that the pH optima under laboratory conditions for degradation of the herbicides dicamba, 2,4-D, dalpon, amitrole and vernolate were 5.3, 5.3, 6.5, 6.5, and 7.5, respectively. The authors assumed these responses were due to the influence of pH on microbial activity (but it could be due to pH effect on sorption). The effect of pH on the ability of *Lipomyces* sp. (a soil yeast isolated from paraquat-treated soil) to degrade paraquat in three media was examined by Anderson and Drew (1972). These researchers found that in malt and mineral-salt medium paraquat was degraded at most pH values but the rates were decreased at extreme pH values (Table 26). In soil extract, the degradation was reduced at extremely acid pH values and was nondetectable at values of pH 8.4 or above.

Table 26. Effects of pH on Ability of Lipomyces sp. to Degrade 10^{-4}M Paraquat in Three Media at 22°C (Anderson and Drew, 1972).

	% Paraquat Degraded After 3 Days in		
pH	Malt Extract	Mineral Salts	Soil Extract
3.6	0.0	0.0	70.0
4.2	12.3	77.1	80.0
4.8	70.5	84.6	100.0
5.4	100.0	87.9	100.0
6.0	93.4	58.3	100.0
6.6	95.4	51.8	100.0
7.2	94.7	35.5	100.0
7.8	90.5	46.9	50.0
8.4	94.1	99.1	0.0
9.0	92.6	68.0	0.0
9.6	49.3	50.8	0.0

The influence of temperature on the breakdown of chemicals in soil is a complex relationship. Increase in temperature generally results in increased rate of volatilization, desorption and leaching. Microbial activity in the soil is also directly influenced by temperature. At low temperatures, the overall transformation of a chemical is slower than at higher temperature (Edwards, 1964; Lichtenstein and Schulz, 1959; Patterson, 1962). DeRose and Newman (1947) tested the persistence of three growth regulators in soil at temperatures ranging from 10 to 30°C and found that persistence was inversely proportional to the incubation temperature. Anderson and Drew (1972) studied the effect of temperature on the degradation of paraquat with a pure culture of a soil yeast (Lipomyces sp.) and found that degradation was decreased at temperatures below 20°C and above 35°C (Table 27). The rates

Table 27. Effects of Temperature on Ability of Lipomyces sp. to Degrade 10^{-4}M Paraquat in Mineral Salts Medium, pH 7.2 in Static Culture (Anderson and Drew, 1972)

Temp. (°C)	Paraquat Degraded After 3 Days (%)	Temp. (°C)	Paraquat Degraded After 3 Days (%)
6	55.5	26	100.0
12	59.6	30	100.0
16	62.6	34	100.0
20	72.3	39	0.0
22	96.1		

of degradation of three triazines and two uracil herbicides were determined by Zimdahl et al. (1970) at temperatures of 13.2 and 31.2°C. Evaluation of the rate constants at the two temperatures revealed always lower degradation rates at the lower temperature (Table 28). Montgomery *et al*. (1972) studied the

Table 28. The Rate of Degradation and Arrhenius Activation Energy of Selected Triazine and Uracil Herbicides Applied to the Soil at 8 ppm (Zimdahl *et al*., 1970)

Herbicide	Rate of Degradation in Reciprocal Months at Storage Temperature (°C)		Arrhenius activation energy (kcals/mole)
	13.2	31.2	
Atrazine	0.19	0.60	10.8
Simazine	0.21	0.55	9.2
Ametryne	0.14	0.26	6.1
Bromacil	0.14	0.19	3.0
Terbacil	0.37	0.59	6.1

degradation of herbicide dichlobenil in soil at temperatures 6.7 and 26.7°C; the half-life of the herbicide at 6.7°C was found to be 28 weeks (+10 weeks lag), and 19 weeks at 26.7°C.

Most degradative reactions catalyzed by soil microbial communities require the presence of oxygen. Anderson and Drew (1972) found no degradation of paraquat under anaerobic conditions in the presence or absence of alternate electron acceptors (e.g., NO_3^- or SO_4^{2-}), whereas under aerobic conditions, degradation was complete within 3 days. The effect of different levels of aeration (obtained by varying the amount of medium in the flask while keeping the agitation constant) on microbial utilization of crude oil has been studied by Jobson et al. (1972). Their results indicated differential utilization of crude oil components at different levels of aeration; there was more rapid utilization of the saturated fraction under conditions of maximum aeration (Table 29). On the other hand, a number of chemicals have been found to be degraded more rapidly under anaerobic soil conditions. Guenzi and Beard (1968) have found fortyfold higher rates of degradation of DDT under anaerobic conditions than under aerobic conditions, although the extent of change in DDT was small. Sethunathan and MacRae (1969) have reported that disappearance of diazinon under submerged conditions was more rapid than that reported in studies under aerobic conditions. Jagnow and Haider (1972) have compared the rates of disappearance of dieldrin in stationary and aerated cultures of several soil microorganisms and found that there was greater release of $^{14}CO_2$ from ^{14}C-dieldrin in stationary cultures than from aerated cultures.

Table 29. Liquid Chromatographic Analyses of Residual Oils from the Aeration Experiment After 5 Days of Growth at 30°C (Jobson et al., 1972)

Oil fraction	Weight (%) of "topped" oil volume				
	Control	250 ml[a]	500 ml[a]	750 ml[a]	1,000 ml[a]
Benzene-soluble asphaltenes	2.5	7.6	4.8	3.3	3.3
Benzene-insoluble asphaltenes	6.8	6.1	6.8	5.0	5.5
Saturates	51.0	35.9	46.8	45.6	47.2
Soluble NSO[b]	8.6	14.4	10.7	10.2	10.1
Insoluble NSO	0.3	1.0	1.0	3.2	1.0

[a] Per 2-liter flask

[b] NSO component: fraction recovered by elution with a 1:1 benzene-methanol mixture. This fraction should contain more polar compounds than those eluted with benzene which yields the aromatic fraction of crude oil.

Willis et al. (1974) have pointed out recently that factors other than mere exclusion of oxygen (e.g., redox potential) may determine the rate of pesticide degradation in anaerobic environments. These researchers investigated the relationship between the oxidation reduction potential (Eh) and the rate of degradation of herbicide trifluralin. It was found that the rate of degradation of trifluralin was much more rapid under more reducing conditions (Figure 31).

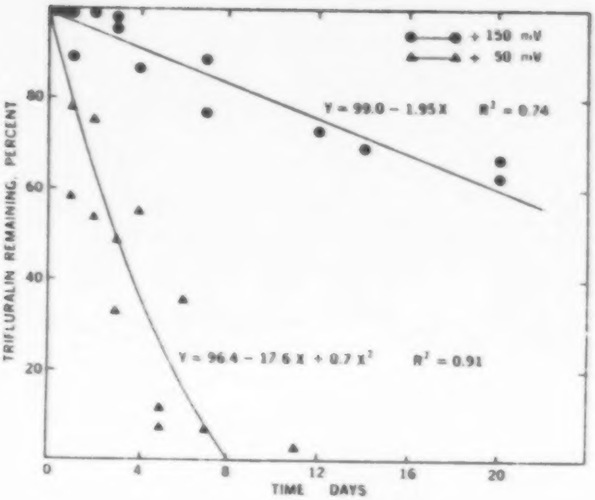


Figure 31. Disappearance of Trifluralin from Soil Suspensions as a Function of Redox Potential and Time. The curve $Y = 99.0 - 1.95X$ was developed from soil suspensions at redox potentials at +450, +250, and +150 mV. The curve $Y = 96.4 - 17.6X + 0.7X^2$ was developed from soil suspensions at redox potentials at +50, 0, and -50 mV. (Willis et al., 1974) Reprinted from Journal of Environmental Quality, 3, 262., American Society of Agronomy.

Soil moisture content can influence persistence of a chemical directly by affecting soil microbial activity, or indirectly by influencing the initial adsorption of the chemical, the rate at which the chemical diffuses into the soil or the availability of the adsorbed toxicant (Gerolt, 1961). DeRose and Newman (1947) reported that plant growth regulators 2,4-D, 2,4,5-T and 2-methyl-4-chlorophenoxyacetic acid disappeared rapidly as the moisture content in the soil was increased. Yaron et al. (1974) reported that the presence of water in soil caused an increase in the rate of loss of the pesticide, azinphosmethyl; the authors explained it on the basis of the fact that biological activity is reduced in a dry environment. The effect of soil water content on the rate of degradation has been summarized by Hamaker (1972).

The calculated rates indicated that the degradation in dry soil may be many fold slower than in moist soil and that the rate tends to level off with higher moisture content with a possible change at saturation values (Hamaker, 1972).

f. External Carbon Source

The role of soil organic matter in degradation of organic chemicals has been discussed in an earlier section. Several researchers in their degradation studies have modified soils with the addition of external carbon sources which differ in type and quantity from the organic matter naturally occurring in soils. This section is devoted to a discussion concerning the influence of external carbon source in the biodegradation test medium on the persistence of environmental contaminants. Similar to other factors which favor soil microbial activity, addition of a readily utilizable carbon source generally enhances the degradation of chemical compounds in soil. The addition of larger amounts of supplemental carbon source, however, frequently results in lowering of the degradation rates, presumably as a result of greater ease with which the organisms are able to utilize the added carbon (Kaufman *et al*., 1968). McClure (1970) has reported increased degradation of the herbicides diuron, monuron, diphenamid, dicamba, chloropropham and atrazine in soils to which nutrient broth had been added. Glucose has been shown to accelerate the degradation of atrazine, diuron, and disodium methanearsonate (McCormick and Hiltbold, 1966; Dickens and Hiltbold, 1967) and parathion (Lichtenstein and Schulz, 1959). Miyazaki *et al*. (1969) have studied the metabolism of the acaricides chlorobenzilate and chloropropylate by pure cultures of *Rhodotorula gracilis*. These researchers found that degradation of chlorobenzilate was stimulated by the addition of sucrose. In contrast, the breakdown of chloropropylate was reduced in the presence of sucrose although the microbial population had increased considerably by the addition of sucrose (Table 30). Kaufman *et al*. (1968) have also reported that addition of organic amendments (e.g., starch, hay, or sucrose) to amitrole-treated soil stimulated microbial activity but reduced amitrole degradation.

Table 30. Radioactive Carbon Dioxide Collected from Culture of *Rhodotorula gracilis* during a 10-day Incubation Period (Miyazaki *et al*, 1969)

Supplement to basal medium (0.5%)	^{14}C-chlorobenzilate		^{14}C-chloropropylate	
	OD[a]	$^{14}CO_2$[b]	OD[a]	$^{14}CO_2$[b]
None	0.022	0.64	0.020	0.10
Sucrose	0.395	1.93	0.355	0.01
Citrate	0.050	1.58	0.027	0.29
α-Ketoglutarate	0.060	1.00	0.067	0.26
Succinate	0.060	0.90	0.056	0.08
Fumarate	0.159	0.45	0.130	0.19

[a] Optical density in 10-fold diluted samples was measured at 420 nm at the end of incubation period.

[b] Percent collected of the originally incorporated ^{14}C-Chlorobenzilate or ^{14}C-Chloropropylate.

2. Correlation Between Laboratory and Field Results

Field studies concerning degradation of organic chemicals in the soil environment have generally been limited to pesticidal chemicals. In most cases, the residue of the applied pesticide has been determined at various intervals by bioassay or other analytical procedure (Burnside *et al*., 1971; Schulz and Lichtenstein, 1971; Lavy *et al*., 1973). In these studies researchers have not attempted to distinguish between the loss of the compound by phenomena such as leaching, volatilization, percolation to subsoil, etc. and due to degradation. Furthermore, parameters such as temperature, moisture, and other environmental conditions are continuously varying in any field experiment, which makes a meaningful comparison between a field study and a controlled laboratory study extremely difficult.

Bro-Rassmussen et al. (1970) studied degradation of eight phosphate insecticides in the laboratory by incubating soil with the test chemical. The disappearance curves of the insecticides were defined by a first order rate constant. When degradation was studied under field conditions, the data failed to fit the first-order rate model since the degradation rates decreased much more rapidly than would be expected for first order kinetics. These authors attributed these differences to the lack of strict control of several parameters (e.g., water content of the soil and temperature) during the duration of the field experiment. However, they found a reasonably good agreement in the laboratory and field results in terms of relative order of persistence of the organophosphorous insecticides tested. Only in the case of two herbicides, bromophos and mercarbam, was the decomposition rate found to be more rapid in the outdoor experiments than in the laboratory experiments. However, the validity of these comparisons is in question, since the field tests had no controls for physical loss.

A number of other comparisons between laboratory and field results are possible. However, the field studies only measure loss of parent compound or soil persistence and, therefore, they do not allow comparisons of degradation.

3. General Discussion of Various Test Methods

Unlike the natural waters, soils are generally richer in microbial content and have, therefore, been used extensively as microbial inoculum without amendment or added microorganisms, in degradation studies. A test method utilizing microorganisms occurring in natural soil approaches more closely the conditions encountered in nature. The use of soil eliminates the need for adjustment of various biodegradation parameters such as microbial population and diversity, nutrient and oxygen concentration, in an attempt to simulate the natural soil environment. The use of soil in a biodegradation test in the laboratory permits the investigator to perform degradation studies under controlled conditions; e.g., where losses due to volatilization, leaching, etc. can be controlled. However, soil, when used in the laboratory, is somewhat removed from many naturally occurring environmental conditions. For example, the moisture content in the laboratory degradation study is usually maintained relatively constant under natural conditions, the moisture content will change substantially. The soil from different geographic locations will vary considerably in terms of physicochemical properties and microbial composition which may introduce serious variations in the results of degradation studies of different laboratories. Furthermore, the presence of a complex medium such as soil will often introduce additional steps in extraction and clean up. Also, the presence of soil may preclude the assay of biodegradation by many analytical procedures. For example, measurement of oxygen consumption with natural soil as the biological material has generally not been used for studying degradation; the reason being, perhaps, the high endogenous rates of soil respiration which may make the interpretation of oxygen uptake data more difficult.

Soils incubated with a test chemical under stationary conditions with or without aeration more closely simulates natural systems than soil suspended in aqueous solution and shaken or incubated under perfused conditions. The former approach allows the investigator to study degradation under aerobic or anaerobic soil conditions. These conditions are known to exist in the natural soil environments and determination of persistence of a compound under all these conditions is essential.

In a number of degradation test methods soil is suspended in water, and therefore, one deals with an aqueous suspension of soil rather than soil at moisture levels generally encountered under natural conditions. The moisture content of the soil can affect degradation of a chemical in many different ways (see Section IV.C.1.e., p.265). When sizable amounts of soil are used with the water, the system simulates flooded soil or sediment conditions. However, in many cases, soil is used as an inoculum in the test procedure and not as a medium for degradation and, therefore, the quantity of soil suspended in the aqueous medium is very small. This might limit the availability of many undefined nutrients present in soil; such nutrients may be essential for certain soil microorganisms to proliferate under the test conditions and degrade the test chemical. The advantage of the method is that in case a dilute soil suspension is used, certain analytical measurements, e.g., disappearance of U.V. absorption, can be made directly on the sample or on the supernatant obtained after centrifugation. The extraction and clean up steps in such cases may be minimal. Furthermore, when the soil suspended in aqueous solution is used in degradation studies, a more uniform distribution of the test chemical may be obtained. This may permit a uniform exposure of the test chemical to the entire microbial population, as well as removal of relatively homogeneous samples for degradation analysis.

Soil perfusion systems are rapid and easy to use but are somewhat difficult to set up and maintain. The test requires that substrate or product appear quantitatively in the perfusate and, therefore, can not be used with the compounds which adsorb to the soil. Furthermore, in soil perfusion systems, one also deals with an aqueous suspension rather than soil and the system is not a good simulation of the soil environment. Soil perfusion systems permit a constant exposure of microorganisms to air, water and the test chemical; subsequently the degradation environment is perhaps more potent than occurring under natural conditions. Kearney (personal communication) has stated that a soil perfusion system is an excellent tool for enriching microorganisms which will degrade the test chemical; however, such a system is unsuitable for use as a routine biodegradation test method due to its unusually higher biodegradation potential and difficulty in handling many units. Recycle trickling filters which operate on the same principle as the soil perfusion system have also been reported to possess higher biodegradation potential compared to other test methods (Swisher, 1970; Cook, 1968). A soil perfusion system has generally been used to study aerobic degradation; the simulation of flooded and anaerobic soil conditions has not been attempted with this test method.

Pure culture and cell-free extract studies are generally considered suitable for elucidation of biochemical pathways and mechanisms of degradation, but are unsuitable for preliminary screening for biodegradability.

According to Wright (1971), organism(s) which can metabolize a compound in pure culture are not necessarily those responsible for its degradation in the soil. Thus, results from pure culture studies may be difficult to extrapolate to the natural conditions. Degradation by pure culture of microorganisms will also fail to allow interaction between natural environmental conditions as well as interaction with other microorganisms. Gunner and Zuckerman (1968) have uncovered a synergistic relationship between two organisms in the degradation of a chemical compound. These researchers found that when the Arthrobacter sp. and Streptomyces sp. were incubated separately, no change was evident in the 'diazinon' molecule. However, when the two organisms were incubated together, extensive degradation of the pesticide occurred. Bollag and Liu (1971) have reported that mixed cultures of investigated microbes were more effective in transforming the herbicide Sevin than pure cultures. Since certain biological reactions are catalyzed by extracellular enzymes, these enzymes may degrade organic compounds in soil. Recently, evidence suggesting that soil enzymes contribute to the breakdown of some organophosphorus insecticides has been presented by Getzin and Rosefield (1968, 1971). If pure cultures of microorganisms are used in degradation studies, the extracellular soil enzymes produced by other microorganisms will be absent.

The greatest advantage in using pure cultures in degradation studies is that complications originating from the complexities and variability of the soil system are eliminated and the extraction and clean up procedure will be simpler. The data obtained from such test methods will be more reproducible than when soil is used as the source of biological material. Other advantages and disadvantages in using pure cultures of microorganisms for studying biodegradation will be similar to those discussed in Section III. D.1., p. 162).

Degradation studies utilizing cell-free extracts have been used by researchers to study the enzymatic mechanisms of degradation. Since the membrane permeability barrier is absent when a compound is exposed to the cell-free extract of a microorganism, one can always question if the compound will be degraded by the intact cell and, if so, will the rates be comparable to those observed with the cell-free extracts.

Among the techniques used to isolate pure cultures of microorganisms for studying degradation of organic chemicals, the enrichment culture technique is the most widely used. This technique provides the investigator with effective microorganisms without having to screen a large number of microbial stock cultures for their degradation ability. In this technique, researchers have generally attempted to enrich microorganisms which can use the test organic chemical as the sole source of carbon and energy. However, sometimes the organisms may be able to metabolize a compound to some extent but not necessarily use it as a sole carbon source for growth (referred to as cometabolic degradation; see Horvath, 1972a). This may account for some failures in isolating organisms by elective culture technique. Beam and Perry (1973) showed degradation of cyclohexane in fertile soil but were unsuccessful in isolating microorganism(s) which could utilize the test chemical as the sole source of carbon and energy. Based on their findings, these researchers proposed that their inability to isolate organisms from soil which can utilize cycloparaffinic hydrocarbons as the sole substrate source cannot be taken as proof that such organisms are not present in the environment. However, the relative importance of cometabolism, as an environmental process, is unknown.

In order to enrich for microorganisms capable of cometabolizing an organic compound, techniques such as analogue enrichment (Horvath, 1972b), and co-substrate enrichment (Horvath, 1973) have been suggested (see Section III.A.3.b., p.107). Using these techniques, investigators have succeeded in isolating pure cultures of microorganisms capable of cometabolizing compounds such as DDT (Wedemeyer, 1967a, 1967b; Focht and Alexander, 1970b), 2,3,6-trichlorobenzoic acid (Horvath, 1971), and 2,4,5-trichlorophenoxyacetic acid (Horvath, 1970). Although researchers have succeeded in isolating microorganisms which cometabolize DDT and trichlorophenoxyacetic acid, it should be emphasized that these compounds are well known to be persistent, thus again questioning the significance of cometabolic degradation from the standpoint of environmental persistence.

Because of the complexities and variability of soil systems, the pathways of degradation have generally been studied with pure cultures or cell-free extracts. The techniques used have been reviewed previously in Section III.D.4.b., p.190).

D. Cost Analysis for Testing Biodegradability of Chemicals in Soil

Degradation studies can be carried out in two phases; (i) to test the overall biodegradability of a compound, and (ii) to identify metabolites and establish pathways of degradation. The analytical method for preliminary testing could be a direct measurement of the parent compound and/or the metabolite, or an indirect method, such as measurement of microbial growth and oxygen consumption, CO_2 evolution, etc. The costs associated with the direct analytical procedure applicable to degradation studies in soil are not different than those described for the aquatic environment. However, the time and effort (or the cost) involved in the extraction and clean up of the sample if soil is used as the degradation medium is generally higher. A detailed biodegradation study is sometimes performed using pure cultures of microorganisms. The cost involved in the intensive study is the same regardless of the environment the pure cultures of microorganisms are isolated from. The cost estimate for detailed biodegradation study in the aquatic environment has been described in Section III., p. 210 and these estimates will also be applicable for detailed study in the soil environment. The cost estimates for preliminary soil biodegradability tests are given in Table 31.

Table 31. Cost Analysis for Preliminary Biodegradability Test in Soil Environment

Biodegradation Test Method	Analytical Method	Equipment Needed and Their Cost	Equipment Cost for the Test	Chemicals, Glassware and Misc. Supplies	Labor Cost @ $60/day	Overhead 125% of Professional Services	Total Cost	Cost/Compd.
Soil incubated with test chemical under stationary conditions (1)	Gas chromatography	Gas chromatograph $8000; Centrifuge $1200	25	75	300	375	775	775
" (20)	"	"	150	800	1800	2250	5000	250
Soil incubated with test chemical under stationary conditions (1)	Radioactive material (measuring loss of parent material)	Scintillation counter, $10,000; Centrifuge, $1200.	30	900 (includes labelled material)	300	375	1605	1605
" (20)	"	"	213	12000 (includes labelled material)	2100	2625	16950	850
Soil incubated with test chemical under stationary conditions (1)	Radioactive material (measure evolution of $^{14}CO_2$; e.g., in a biometer flask).	Scintillation counter, $10,000	15	930 (includes labelled material)	180	300	1510	1510
" (20)	"	"	100	13000 (includes labelled material, biometer flasks)	1200	1500	15800	790
Soil incubated with test chemical under stationary conditions (1)	Bioassay	-	-	70	180	225	475	475
" (20)	"	-	-	500	1200	1500	3200	160
Soil suspended in aqueous solution (1)	Gas chromatography	Gas chromatograph, $8000; Centrifuge, $1200.	25	75	300	375	775	775
" (20)	"	"	127	800	1500	1875	4300	215
Soil suspended in aqueous solution (1)	Radioactive material (measure loss of parent compound)	Scintillation counter, $10,000; Centrifuge, $1200.	30	750 (includes labelled material)	300	375	1455	1455
" (20)	"	"	185	12000 (includes labelled material)	1800	2250	16235	810
Soil suspended in aqueous solution (1)	Radioactive material (measure evolution of $^{14}CO_2$)	Scintillation counter, $10,000	20	800 (includes labelled material, biometer flask or gas train assembly)	240	300	1360	1360
" (20)	"	"	100	13000 (includes labelled material, biometer flasks or gas train assembly)	1200	1500	15800	790
Soil suspended in aqueous solution (1)	U.V. Absorption	Spectrophotometer, $3,000; Centrifuge, $1200.	8	50	120	150	328	328
" (20)	"	"	40	300	600	750	1690	85
Soil suspended in aqueous solution (1)	Colorimetric (e.g., chloride ion release)	Spectrophotometer, $3,000; Centrifuge, $1200.	12	75	180	225	495	495
" (20)	"	"	60	400	900	1125	2485	125
Soil perfusion test (1)	Colorimetric (e.g., chloride release)	Spectrophotometer $3,000; Centrifuge, $1200.	28	100	420	525	1073	1073
" (20)	"	"	200	800	3000	3750	7750	388
Soil perfusion test (1)	Gas chromatography	Gas chromatograph, $8000; Centrifuge, $1200	50	100	600	750	1500	1500
" (20)	"	"	305	1200	3600	4500	9605	480

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V. PHOTOCHEMICAL AND CHEMICAL ALTERATIONS

A. Degradation of Chemicals in the Atmospheric Environment

1. Introduction

Atmospheric transport is a major route for the distribution of chemical contaminants of the environment (Risebrough et al., 1968; Risebrough, 1969; Stanley et al., 1971; Compton et al, 1972; Wood, 1974). In addition, for many of the prominent air pollutants (e.g., SO_x, NO_x, CO, hydrocarbons, particulate matter, photochemical smog, etc.), the atmospheric route provides the predominant route of exposure to man, and other biota. Thus, an understanding of the atmospheric chemistry of these contaminants is extremely important to considerations of their persistence and fate in the environment. Unlike the alterations discussed in other sections of this report, atmospheric alterations of chemical contaminants are entirely non-biological.

Substances emitted to the atmosphere, as with other medium, may remain as the parent compound or be converted to more or less objectionable products. The variety of physical and chemical influences on a chemical contaminant is extremely complex and reviews of the reactions and mechanisms of the assimilation process are presented elsewhere (Haagen-Smit and Wayne, 1968; Altshuller and Bufalini, 1971; Leighton, 1961). Presented here will be only a brief discussion of the unique conditions to which a contaminant is exposed in the atmospheric environment and the experimental procedures that are used to study a chemical's reactions in the atmosphere.

The atmospheric media provides an excellent matrix for photochemical or oxidative alterations of chemical contaminants. The intensity and wavelength (>290 nm in the lower atmosphere) of sunlight are capable of initiating

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a variety of reactions. For example, the energy provided by sunlight is able to break carbon-carbon and carbon-hydrogen bonds, cause the photodissociation of nitrogen dioxide to nitric oxide and atomic oxygen, and photolytically excite a relatively large number of oxygen molecules due to their high concentration. This relatively high concentration of oxygen (20.9% V/V) makes it one of the most important participants in various reactions with air pollutants since the rates of reactions are concentration dependent (Haagen-Smit and Wayne, 1968). Similar reasoning can be used for reactions with water vapor (0.1 - 5% V/V) and carbon dioxide (.03 - 0.1% V/V).

Conditions in the upper atmosphere are even more vigorous. Above heights of 50 miles, oxygen exists almost exclusively in the atomic form. Between 10 and 20 miles above the earth a region of high ozone concentration exists. Above this level the energy of the incident sunlight is much higher (<290 nm) because the ozone level is not available to absorb the high energy light. It is generally believed that, through turbulence and diffusion, contaminants from the lower atmosphere will be exposed to the upper atmosphere and this may be a major sink for environmental contaminants.

Physical processes may also be important to both fate and the residences time of chemicals in the atmosphere. Adsorption of substances on particulate matter and subsequent gravitational settling appears to provide a major source of pesticide (and presumably other chemicals) removal from the air (Risebrough _et_ _al_., 1968). A "washout" mechanism caused by falling drops of rain may also be important, especially for water soluble materials. In addition, the adsorption of chemicals on particulate matter may drastically effect the photochemical reaction rate (Klein and Korte, 1971).

Thus, the atmospheric conditions that need to be simulated in the laboratory for the evaluation of a chemical's persistence and fate are extremely complex. A variety of approaches have been utilized.

2. Techniques Used for Determining Atmospheric Degradation

Most studies of atmospheric chemistry have concentrated on processes which have physiological or toxicological significance. These have included processes important to smog formation, eye irritation, oxidant levels, etc. For example, the reactivity of hydrocarbons are often studied in relation to the rate of oxidation of nitric oxide to nitrogen dioxide, an important first step that results in photochemical smog formation. Rarely is a chemical species studied just to determine its chemical reactions in the atmosphere. The interpretation of results varies considerably, depending upon whether one is evaluating long-term (e.g., trace environmental contamination) or short-term phenomena (e.g., smog formation). For example, reactive hydrocarbons in the atmosphere are considered extremely undesirable because of their contribution to the formation of smog. This is analagous to dissolved oxygen depletion in water by high BOD compounds. The techniques which are discussed in this report are considered mostly for their ability to assess a chemical's persistence and fate rather than to predict air pollution consequences.

a. Long-Path Infrared Cells

Long-path infrared cells have frequently been used to study the atmospheric photooxidation of hydrocarbons and nitric oxide. Stephens and coworkers (Hanst et al., 1956; Stephens, 1958) were among the first to use long-path infrared spectroscopy (LPIR) in the study of air pollution chemistry. The apparatus they used consisted of a 3 meter, 18 inch diameter stainless steel tube which contained a three mirror multiple-reflection system. The total path-length was 216 meters (72 passes). The cell was connected to a single beam infrared spectrometer.

Tuesday and Glasson (Tuesday, 1963; Glasson and Tuesday, 1970a, 1970b, 1970c, 1971) used a similar 3 meter stainless steel long-path cell, but with only 40 passes (total path-length 120 meters). Irradiation was supplied by a number of eight-foot black light fluorescent bulbs (F96 - 8/BL) mounted inside the long-path cell. The cell was attached to a modified Perkin-Elmer Model 21 Infrared Spectrometer. A full description of the apparatus is given by Tuesday (1961).

The experimental procedure consisted of first determining the actinic light intensity of the lamps by measuring the rate of nitrogen dioxide photodissociation at very low concentrations in nitrogen gas. Because the quantum yield is close to unity over the spectral region of interest, this is a common method of determining the light intensity in order to compare it to the intensity of sunlight (Tuesday, 1961). In all of Tuesday and Glasson's work the light intensity was 0.27 min.^{-1}

$$K_d\ (NO_2) = \ln \frac{(NO_2)\ \text{init.}}{(NO_2)\ \text{final}} = \text{rate min}^{-1}$$

The long-path cell was evacuated to a pressure of less than 10μ Hg, and known pressures of nitric oxide and nitrogen dioxide were expanded into the cell from an attached glass vacuum system. Hydrocarbons with high vapor pressures at room temperature were also expanded into the cell from the glass vacuum system while hydrocarbons with low vapor pressures were added by a 10-μl syringe to an electrically heated stainless steel manifold containing several high vacuum values and a silicone rubber septum. The hydrocarbon was expanded from the manifold into the cell and the manifold was flushed with nitrogen. Nitrogen was then added to the long-path cell to about 600mm of Hg. After the addition of oxygen (155 mm Hg), the final pressure was brought to 760 mm. of Hg with a small amount of nitrogen. The concentrations of hydrocarbons in most cases varied from 0.25 to 5.0 ppm, with initial concentrations of nitric oxide and nitrogen dioxide of 0.38 ppm and 0.02 ppm, respectively. Quite often these concentrations were varied when the investigators were studying the effect of nitric oxide concentration on hydrocarbon photooxidation or vise versa. These concentrations are well within those existing in polluted atmospheres [3 to 5 ppm hydrocarbon expressed as ppm carbon and 0.2 to 0.6 ppm nitrogen oxides (Korth _et al_., 1964)]. The amount of thermal oxidation of nitric oxide in the cell prior to irradiation was less than 1% at the concentrations used (Glasson and Tuesday, 1970a). The concentrations of nitrogen oxides, hydrocarbons and photochemical products were measured by scanning the infrared spectra.

A more recent study used LPIR for an assessment of the effects of fluorocarbons on the phenomena of photochemical smog (Japar _et al_., 1974). These researchers used two (reference and sample) standard long pathlength cells which were internally coated with FEP (fluorinated ethylene-propylene) Teflon in order to decrease any effects due to wall reactions. The cell optics were adjusted to obtain a total pathlength of 40 meters. Irradiation was supplied by an external

17.5" arc-length, 1200 watt Hanovia medium pressure mercury arc which was surrounded by a double-walled quartz jacket cooled with distilled water. The light passed through six 3" x 3" irradiation ports placed along the length of the cell. The port consisted of 1/4" thick glass which passed only wavelengths longer than 310 nm. The actinic intensity of the lamp measured as nitrogen dioxide dissociation in nitrogen was 0.131 min.^{-1} This intensity was noted to be comparable to a Los Angeles noontime measurement of 0.37 min.^{-1}

The reaction mixtures were expanded into the LPIR cells from an attached glass vacuum system. Concentrations of fluorocarbons, nitrogen dioxide and olefin varied from 5 to 8 ppm, 10.5 to 12.5 ppm and 15 to 34 ppm, respectively. The LPIR cells were pressured to slightly less than 1 atmosphere with compressed air which was passed through a Matheson #450 Gas Purifier and a 3" diameter, 2.5' Dierite column to remove any oil and most of the water present in the air. Concentrations of the fluorocarbon, olefin, and nitrogen dioxide were measured by scanning the infrared spectra before, during, and after irradiation through sodium chloride windows in the LPIR cells.

The Environmental Protection Agency at Research Triangle Park, N.C. (Bufalini, personal communication) is presently using a 30 ft. long (six sections) LPIR made of pyrex glass with Teflon joints. Fourier transform infrared spectrometry is used which allows computer comparisons of results and instantaneous determination of the spectra. Concentrations as low as ppb levels can be used. The fluorescent lights are mounted on the outside of the reaction chamber.

b. Plastic Containers

A popular method for studying the atmospheric chemistry and photochemistry of both artificial and natural atmospheric samples is the use of plastic containers. The common bag material is either FEP (Fluorinated ethylene-propylene) Teflon, Tedlar or Mylar plastic.

Saltzman and coworkers (1966) used this techniques to evaluate the stability of sulfur hexafluoride, bromotrifluoromethane, and octafluorocyclobutane for use as meteorological tracers. The gas being evaluated (SF_6 - 3 to 8 ppb; $BrCF_3$ - 35 to 100 ppb; C_4F_8 - 100 to 500 ppb) was put into the bag with air from a cylinder and small amounts of some of the common reactive pollutants of the atmosphere (H_2O, O_3 - 5.9 ppm, SO_2 - 5 ppm, automobile exhaust 2.8% and H_2S - 5 ppm)were added. Sufficient water to provide a saturated mixture was injected into the bag before addition of the gas mixture. Irradiation studies were carried out with fluorescent black-lights (GE F42T6BL) with 100-liter FEP bags (good transparency in the ultraviolet region). For studies where ozone was incorporated, Mylar bags were used. A control run without the pollutants showed that the gases were slowly lost and this was attributed to diffusion through the 0.002 thick plastic film. No decomposition within experimental error was reported for any of the tracer gases. The possibility of washout by rain fall was checked by placing a bag saturated with water vapor into a 3°C refrigerator. The cold air sample was then drawn into another bag and analyzed. Analysis in all cases was provided by gas chromatograph (no preconcentration) with an electron capture detector.

Altshuller et al. (1967) used FEP plastic containers to study the photooxidation of hydrocarbons in the presence of aliphatic aldehydes. The reaction mixtures of olefin and aldehyde diluted with air (150 liters) were irradiated with sunlight-fluorescent lamps (maximum intensity at 310 nm) and with natural sunlight. Rates of decomposition of the olefin were determined by gas chromatography using a flame ionization detector.

Kopczynski and coworkers (Altshuller, et al., 1970b; Kopcznyski et al., 1972) have used plastic containers to measure photochemical reactions of actual atmospheric samples. In the more recent study, they used atmospheric samples collected in a 300 liter Tedlar bag in the early morning (particulates not removed). Irradiation with sunlight was begun as soon as the sample was collected. Duplicate samples were run and a comparison was made between full sunlight and 58% attenuated sunlight. An important step in the procedure was the conditioning of the bag by irradiating an air sample the day before an experimental run. Analysis of the hydrocarbons was provided by low temperature gas chromatography using a preconcentrated sample and flame ionization detector(GC-FI).

Gay and Bufalini (1971) also used plastic containers in their study of nitrogen balance of irradiated hydrocarbons in the presence of oxides of nitrogen. They used a 100 liter FEP Teflon bag irradiated with GE-F42-T6 black lamps with an energy maximum at 366 nm.

c. Glass Flask Reactors

A number of researchers have used glass flask reactors to study atmospheric chemical reactions. The size of the reaction vessel has varied from 100 ml spherical vessels to 72 liter borosilicate flasks.

Wei and Cvetanovic (1963) in a study of the vapor phase reaction of ozone with olefins used two matched 100 ml spherical reaction vessels made of pyrex glass. These were incorporated into a conventional high vacuum line. The reaction vessels were connected to a gas chromatograph in order to facilitate analysis. A somewhat similar apparatus was used by Wiebe et al. (1973) to study the photolysis of methyl nitrite in nitric oxide, nitrogen dioxide, and oxygen. They used a 500 ml spherical pyrex flask connected in a vacuum line. Irradiation was supplied by a mercury arc lamp (pyrex filters out light below approximately 300 nm). The reaction mixture was analyzed by a mass spectrometer which was connected by a capillary tube to the reaction vessel. Relatively high concentrations of reactants were used (O_3 - 2 mole%; olefins - 20 mole%). The same apparatus was used by Shortridge and Heicklen (1973) to study the chain mechanisms important to the photochemical conversion of NO to NO_2. These authors used a quartz vessel and a variety of light sources including a low pressure mercury lamp (mostly 253.7 nm). The reactions were studied at low pressure (25 torr) and high reactant concentration.

Gay and Bufalini (1971) used two different sized borosilicate flasks as well as a 100 liter plastic bag to study the nitrogen balance of irradiated hydrocarbon and oxides of nitrogen mixtures. A 22 liter borosilicate flask, having a silvered outer surface was used to determine if molecular nitrogen is a product of photolysis. A mercury lamp (Hanovia #679A36) fitted in a double-walled, water cooled, immersion well was used as a light source. Initial concentrations of 60 ppm ethylene and 15 ppm NO_2 in helium or oxygen were used. A similar apparatus, although equipped with a quartz immersion well (Corex filter-cut off at 260 nm), was used by Bentley et al. (1972) to study the photolysis of dimethyl sulfide in air (68 mg/l). These conditions were used to determine the applicability of photochemical methods of controlling odor problems in the paper industry.

The larger borosilicate flask used by Gay and Bufalini (1971) was a 72 liter flask. This reaction vessel was placed inside an irradiation chamber described by Altshuller and Cohen (1963). Irradiation is provided by GE-F42-T6 black lamps with an energy maximum at 366 nm. The light intensity of the system measured in terms of nitrogen dioxide dissociation was 0.4 min.^{-1} Before each experimental run, the flask was washed with aqueous cleaning solution, 300 ml of acetone, and large amounts of distilled water. The flask was allowed to dry and then equipped with a large magnetic Teflon-finned stirrer and a top with several glass and Teflon lines which served as inlets and exhausts, some of which were connected to gas chromatographs. The flask was then evacuated and refilled with air and the pollutants to be studied. The relative humidity of the tank air was 5%.

Bufalini et al. (1972) compared the 72 liter borosilicate flask to a 335 ft^3 smog chamber (Rose and Brandt, 1960) in a study of reasons for poor interlaboratory comparisons of various smog chambers. The 72 liter flask was irradiated in the chamber described above with different light sources (GE F-40-BLB blacklights, NO_2 $K_d = 0.50\ \text{min}^{-1}$ and Westinghouse FS-40 sunlights, NO_2 $K_d = 0.14\ \text{min}^{-1}$.) The rate of tetramethylethylene photolysis and ozone formation from biacetyl was studied in "clean" and "dirty" flasks. The dirty flasks were ones that were not cleaned with dilute sodium hydroxide solution before each run.

Laity and Maynard (1972) studied the reactivities of gasoline vapors with three experimental apparatus: (1) 400-liter stainless steel chamber, (2) a 235-liter glass vessel, and (3) a 23-liter glass flask. The large glass vessel is a capped, borosilicate-glass, spherical vessel which is placed in a metal box for irradiation. The source of irradiation in all cases is blacklight fluorescent lights. The light intensity is 0.4 min^{-1} (K_d NO_2, 2 ppm in N). Before each run, the reaction vessels are cleaned by ozone treatment and evacuation (10^{-2} to 10^{-3} torr, 36 hours, ∿ 60°C).

Stephens and coworkers (Stephens, 1973; Stephens and Burlenson, 1967) have used 20 and 50 liter borosilicate carboys to irradiate ambient air samples to study hydrocarbon reactions in air. Irradiation was carried out with both natural sunlight and artificial light (blacklight fluorescent lamps NO_2 K_d 1/2 life = 1.3 min) using a static technique with the 20 liter carboy. When studying aromatics, where the loss of reactant by absorption on the wall is possible, Stephens (1973) used a stirred flow dilution technique. With this technique the air sample in the 50 liter carboy is continuously diluted with dry, hydrocarbon-free, make-up air during the irradiation. No difficulty with adsorption of aromatics was noted. Analysis was provided by gas chromatography combined with a trapping technique in order to make 0.1 ppb detection possible.

A 72 liter borosilicate glass flask of special design has been used by Crosby and Moilanen (1974) to study the vapor phase photolysis of aldrin and dieldrin. The apparatus is depicted in Figure 32. The flask is painted dull black on the outer surface. The light from the lamp (E in Figure 32) is passed through the inside of a 59 mm id (27 cm^2 in area) borosilicate glass tubing painted dull black on both the inner and outer surface. The light exits through an unpainted circular area (c) 15 cm in diameter. The removable light trap (D) could be replaced with a 1 liter spherical flask silvered on the interior. A 3 mm-thick borosilicate glass disc (b) prevents escape of vapor and filters out UV wavelengths below 300 nm. Inlet and outlet tubes are used only as vents. The lamp is either a 100W low-pressure mercury lamp (254 nm filtered out, 365, 410, and 440 line) or a 275W RS-sunlamp. In the procedure, the compound to be studied was formed into a thin solid film (hexane evaporation) on a watch glass which is then placed into the bottom of the reaction chamber and warmed to 35°C. After allowing the chemical vapor to evaporate into the chamber (several hours), irradiation is begun. After irradiation for an arbitrary period (45 - 168 hours), the reactor is dismantled and washed with hexane. By comparing results with and

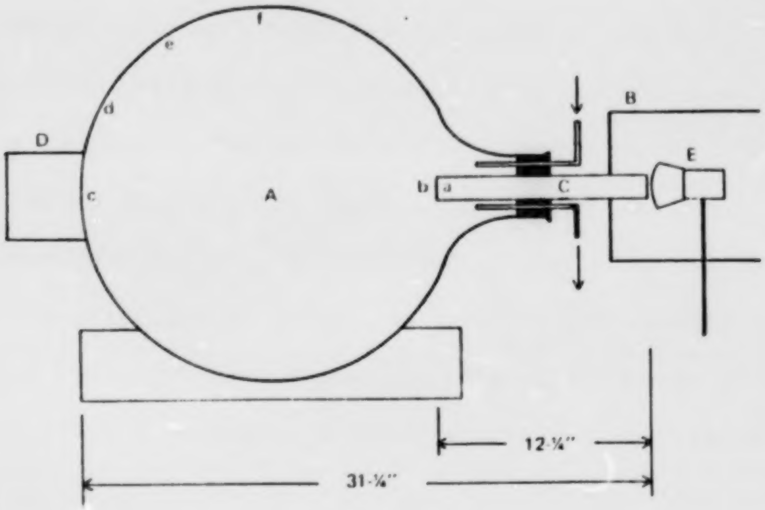

Figure 32. Schematic Diagram of Vapor-Phase Photoreactor
(Crosby and Moilanen, 1974)
Courtesy of Springer-Verlag

without the mirror (varying the light impinging on the inner surface of the reaction chamber), the effect of wall reactions, if any, could be determined. A completely dark control is also run in order to provide evidence that non-photochemical reactions are not taking place.

Laity (1971) also used a spherical 22 liter borosilicate glass flask fitted with a glass cap and containing an all-glass magnetically driven stirrer to compare results using blacklight fluorescent lamps with results using natural sunlight.

Urone et al. (1968) used a 2-liter reaction flask in the study of sulfur dioxide reactions in air. A mixture of sulfur dioxide tagged with $S^{35}O_2$ in clean, dry air was prepared in 20-liter Mylar bags. These were then transferred to one or more calibrated, 2-liter quartz or borosilicate flasks. Triple-distilled water was added to the flask with a 50 μl syringe to obtain the desired relative humidity and the flask was kept in the dark overnight. Just before irradiation nitrogen dioxide was added to the flask. Irradiation was provided by a bank of 30 x 1.5 cm intermediate pressure ultraviolet lamps, coated to give a spectral distribution from 310 to 420 nm with 90% of the intensity near 350 nm. The intensity was measured by potassium ferrioxalate actinometry and found to be "about seven times the noonday sunlight in the same wavelength region." The rate of sulfur dioxide reaction was measured in the presence and absence of hydrocarbons and inert solids, such as sodium chloride.

d. Smog Chambers

There are three major drawbacks to the previously mentioned experimental apparatus for studying atmospheric reactions: (1) their small size allows the possibility of heterogenous reactions, (2) ratio of volume to surface allows significant radical termination at the walls, and (3) in some cases the contaminant concentrations have to be unrealistically high in order to provide analytical precision (Doyle, 1970). In order to reduce these difficulties, many researchers have resorted to large photochemical "smog chambers," so named because of their unique capability in studying the phenomena of photochemical smog. The large size of the chamber also allows for simultaneous evaluation of physiological effects such as eye irritation and plant damage.

Most of the smog chambers reported in the literature can be operated in a dynamic or static mode. The dynamic procedure requires the continuous introduction of reactants and is thought to more closely simulate the dynamic characteristics of the atmospheric environment. It is particularly suited to studies which required large analytical samples. The static procedure consists of batch introductions of reactants into the chamber and because of its simplicity and precision, it is more predominently used (Dimitriades, 1967).

(i) Rose and Brandt Smog Chamber

One of the earliest chamber facilities reported in the literature and one still used by EPA (Bufalini et al., 1972) is the enviromental irradiation test facility of Rose and Brandt (1960). The facility consisted of five major components: a dynamometer unit, a dilution air purification unit, exhaust dilution assembly, irradiation chambers, and an exposure facility to evaluate air pollution effects of the irradiated pollutants (See Figure 33). The dynamometer unit ran an internal combustion engine in a way representative of various driving patterns. The exhaust gas was diluted with a clean air supply (cleaned with particulate and charcoal filters) which was maintained at temperatures between 60 and 100°F and at a relative humidity from 35% to 90% by employing a refrigeration air conditioning system. The hydrocarbon concentration was several ppm. The two irradiation chambers, which could be operated in series or parallel, were constructed of aluminum with Mylar windows and had a volume of 335 ft^3 each.

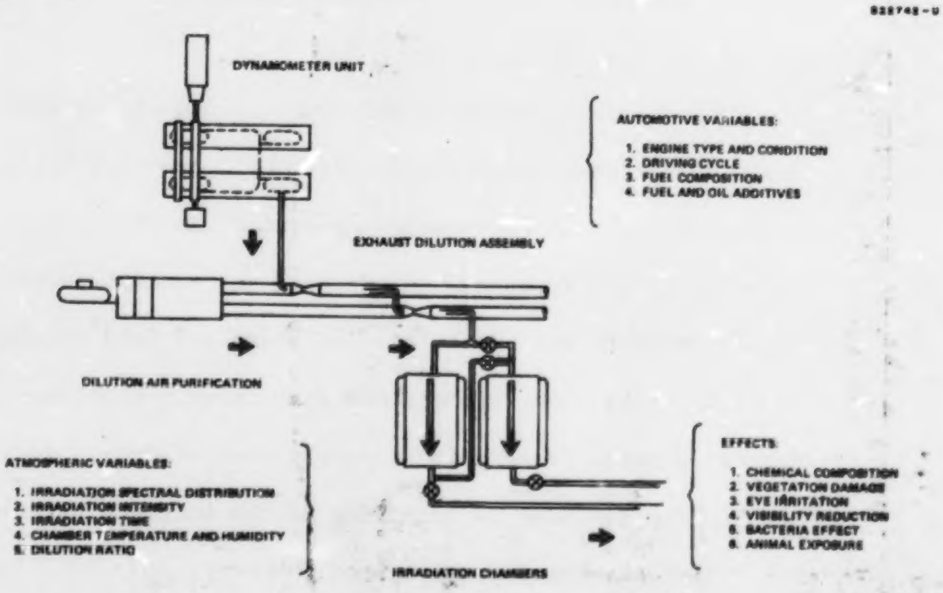
822742-U
DYNAMOMETER UNIT
AUTOMOTIVE VARIABLES:
1. ENGINE TYPE AND CONDITION
2. DRIVING CYCLE
3. FUEL COMPOSITION
4. FUEL AND OIL ADDITIVES
EXHAUST DILUTION ASSEMBLY
DILUTION AIR PURIFICATION
ATMOSPHERIC VARIABLES:
1. IRRADIATION SPECTRAL DISTRIBUTION
2. IRRADIATION INTENSITY
3. IRRADIATION TIME
4. CHAMBER TEMPERATURE AND HUMIDITY
5. DILUTION RATIO
IRRADIATION CHAMBERS
EFFECTS:
1. CHEMICAL COMPOSITION
2. VEGETATION DAMAGE
3. EYE IRRITATION
4. VISIBILITY REDUCTION
5. BACTERIA EFFECT
6. ANIMAL EXPOSURE

Figure 33. Environment Irradiation Test Facility
(Rose and Brandt, 1960)
Courtesy of Air Pollution Control Assn.

Circulation in each chamber was maintained by two tube-axial fans. The chambers were designed to approach ideal dilution performance (concentration of output gas would be representative of the entire volume). The average leak loss from one chamber was 0.004 cubic feet/min (cfm). Irradiation was applied to each chamber by two banks of 70 fluorescent tubes (black, warm white, and blue) in order to simulate solar irradiation from 290 to 450 nm. The exposure time was varied by changing the flow rate (20 to 100 cfm).

When Bufalini and coworkers (1972) used the chamber, they determined the light intensity to be 0.40 min^{-1} in terms of NO_2 dissociation. They studied the oxidation of nitric oxide in the chamber and noted an oxidation rate much faster than theoretical which they attributed to chamber wall contamination.

(ii) Wayne and Romanovsky Smog Chamber

Wayne and Romanovsky (1961) used a 1000 ft^3 chamber in a study of the photooxidation of automobile exhausts using six different fuels. A dynamometer apparatus similar to that of Rose and Brandt (1960) was used to generate the exhaust gases. The irradiation chamber was constructed of glass and held together with an aluminum frame. Forty-eight 400 watt mercury-arc lamps externally mounted provide irradiation through pyrex windows. An internally mounted fan provides rapid mixing of the entering materials.

(iii) Korth, Rose and Stahman Smog Chamber

Korth and coworkers (1964) in studies conducted at the Taft Sanitary Engineering Center again used a five component facility in a dynamic study of automobile exhaust gases.

The irradiation chamber is a 335 ft^3 vessel, presumably the same as used by Rose and Brandt (1960). However, the light intensity was increased (present configuration, Figure 34) to be more representative of sunlight in the Los Angeles area at a zenith angle of 20 degrees (sunlight curve #2, Figure 34).

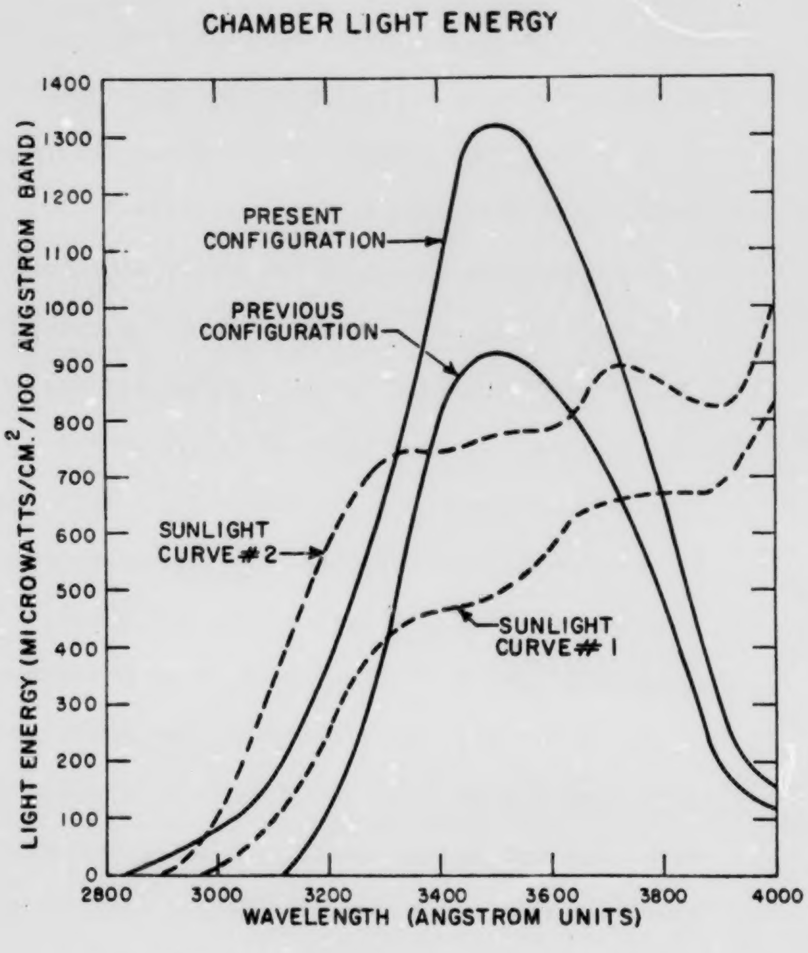

Figure 34. Chamber Light Energy for Korth *et al*. (1964) Chamber
Courtesy of Air Pollution Control Assn.

Altshuller and coworkers (Altshuller et al., 1969a,b; 1970a) have made extensive use of the Korth et al. (1964) chamber. Although a few experiments have been conducted under dynamic conditions, most of their experiments have used a 6 hour, static-irradiation period. In the 1970 study, a temperature of 31 ± 1° and relative humidity of 50% was maintained. These authors studied the photochemical reactivities of paraffinic and aromatic hydrocarbons with low concentrations of nitric oxide. Kuntz et al. (1973) used the chamber to study the photoreactivity of benzaldehyde-NO_2 and benzaldehyde-hydrocarbon-NO_x mixtures. They cleaned their make-up air by passage through activated charcoal and particulate filters.

(iv) Bartlesville Petroleum Research Center Smog Chamber

Two irradiation chambers with volume capacities of 64 and 100 ft^3 have been used for air pollution research by the Bartlesville Petroleum Research Center of the U.S. Bureau of Mines (Dimitriades, 1967). The 64 ft^3 chamber was constructed so that the gaseous contents only contacted aluminum and a 5 mm Teflon film. The Teflon film was preferred over the previously used Tedlar (polyvinyl fluoride) because it is less permeable to water vapor and more transparent to ultraviolet light (see Figure 35). The chamber is equipped with nine sampling ports and is usually run using the static procedure. There are two fans mounted inside for internal circulation. The 100 ft^3 chamber was similar to the 64 ft^3 chamber except that it

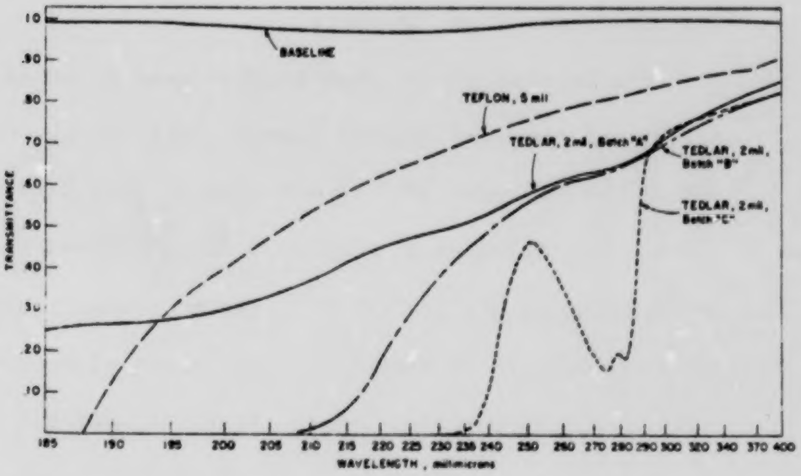

Figure 35. Spectra of Teflon and Tedlar Films in the UV Region (Dimitriades, 1967)
Courtesy of Air Pollution Control Assn.

had pyrex windows. The ozone half-life time at 1 ppm in the small and large chambers with the lights on was 3.6 hr. and 6 hr., respectively. The irradiation system consisted of a number of fluorescent lamps designed to produce a light intensity similar to natural sunlight. The K_d NO_2 was 0.38 min^{-1}. In order to provide analytic samples, 0.7 liters/min was withdrawn from the chamber and replaced with pure N_2 (dilution rate 2.3%/hr). Using this apparatus, Dimitriades (1967) studied the effects of background reactivity (chamber reactivity), temperature, and humidity on hydrocarbon-NO_x reactions.

(v) Stainless Steel Chambers

The General Motors smog chamber used by Heuss and Glasson (1968) and the smog chamber used by Laity et al. (1973; Laity & Maynard, 1972) were both constructed of stainless steel. The GM chamber consisted of a cylinder 9 ft. in diameter and 5 ft. high with an internal volume of 298 ft^3. Spaced symmetrically throughout the chamber were 19 vertical borosilicate glass tubes which contain the irradiation lamps; the K_d NO_2 was 0.4 min^{-1}.

The chamber used by Laity et al. (1973) consisted of a polished stainless steel cylinder, 4 ft. long and 2 ft. in diameter (397 liters-14 ft^3) with blacklight fluorescent lamps mounted inside. Temperature was controlled by circulating liquids around the chamber and between experiments the chamber was cleaned by evacuation and heating (50 - 60°C) overnight. The half-life of ozone (1 ppm) under irradiation varied from 1.3 to 2.5 hours and the intensity of the light was 0.4 min^{-1} (K_d NO_2). The results from the chamber were compared to results using two glass vessels (235 ℓ and 23 ℓ). The following experimental conditions were used: 5 hrs. irradiation, temperature 32°C, ∿ 1 ppm test chemical, 0.6 ppm nitrogen oxides (0.57 ppm NO, 0.03 ± 0.02 NO_2) and 20% relative humidity.

(vi) Stanford Research Institute Smog Chamber

The SRI smog chamber reported by Doyle (1970) is an elaborate facility. The photochemical reaction chamber is mainly constructed of rolled sheets of 7/32 inch Pyrex (transparent down to 300 nm) with some cast aluminum sheet panels. All interior surfaces are coated with Teflon. Sonic jet-type pumps are used to stir the chamber contents (avoids difficulties from impellers). The chamber configuration depicted in Figure 36 has an internal volume of 7.6 m^3 (269 ft^3). Irradiation is supplied by fluorescent lamps uniformly distributed above the exterior of the chamber. The number and types of lamps were chosen to simulate sunlight intensity from 300 to 600 nm. The longer wavelengths were simulated because the chamber was designed to study compounds that might absorb in that region. The leakage rate from the chamber was 1.5%/hr. and the ozone half-life in the irradiated chamber was 6.7 hr. The temperature can be controlled down to 27°C with the lights on and the make-up air is humidified to the desired level (usually 35% at 1 atm. and 25°C). Sample ports are built in

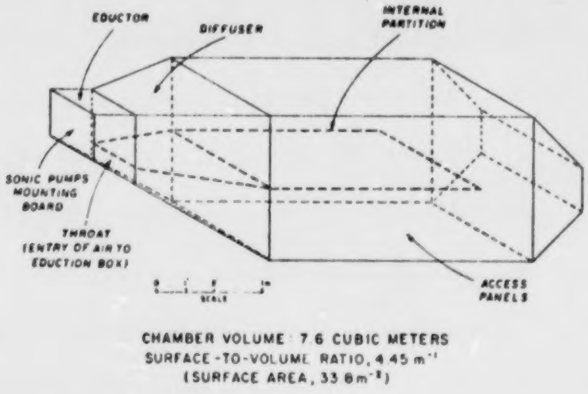
EDUCTOR
DIFFUSER
INTERNAL PARTITION
SONIC PUMPS MOUNTING BOARD
THROAT (ENTRY OF AIR TO EDUCTION BOX)
ACCESS PANELS
0
1m
SCALE
CHAMBER VOLUME 7.6 CUBIC METERS
SURFACE-TO-VOLUME RATIO, 4.45 m⁻¹
(SURFACE AREA, 33.8m⁻²)

Figure 36. SRI Smog Chamber
(Doyle, 1970)
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the chamber for destructive analysis and the optics of a LPIR (40 m path length) are inserted into the chamber through a Teflon-film diaphragm, thus allowing non-destructive analysis.

A major effort was made to purify the make-up air. This was accomplished using catalytic combustion over platinum at elevated pressure (5 to 8 atm.) and temperatures (600°C). The pure air supply had the following analysis: <0.5 ppm CO, 0.1 - 0.3 ppm NO_2, and some organic material as analyzed by GC-FI. An 8 hr. irradiation of the pure air yielded no detectable oxidant concentrations and little change in the nitrogen oxide concentrations.

(vii) Battelle Memorial Institute Smog Chamber

Wilson et al. (1972a,b) have reported the use of the Battelle 610 ft^3 smog chamber made of polished aluminum with Teflon FEP window for the study of a variety of pure organic compounds (α-pinene, cyclohexene, 1-heptene, and toluene). The initial conditions were the following: temp. 88-90°F, relative humidity 39-43%, hydrocarbon - 10 ppm, NO - 0-1 ppm, and NO_2 - 1-2 ppm. Analysis of reaction products was provided by gas chromatography interfaced with mass spectrometry (GC-MS).

Both the SRI smog chamber and the Battelle smog chamber have been used in a comprehensive study of air pollution problems resulting from organic solvent use (Levy, 1973).

3. Analytical Procedures

Analytical procedures have considerable influence upon the reaction conditions used and the results obtained in studies of chemical reactions in the environment. If the sensitivity of the method is low, it may require unrealistically high concentrations or large samples for analysis. Measurement of the reactivity of hydrocarbons and solvents determined by NO loss, NO_2 formation, oxidant formation, hydrocarbon loss, eye irritation, or plant damage can give drastically different results. This section briefly discusses and evaluates the analytical techniques that are used most commonly in atmospheric studies.

a. Long-Path Infrared Spectrometry

When LPIR cells are used as reaction vessels, or when smog chambers are equipped with LPIR windows (Doyle, 1970), the concentrations of the reactants and products are followed by scanning the infrared spectrum. Theoretically any chemical that absorbs between 2 and 15 microns can be monitored. However, in practice a compound must have sharp, high extinction coefficient, diagnostic peaks which do not overlap other absorption bands and this somewhat limits the number of chemicals that can be studied in any one experimental run. These peaks will vary depending upon the chemicals being studied. However, interfering peaks will in most cases be the same. Some of the interfering peaks that have been reported are listed in Table 32.

Table 32. Interfering Infrared Absorption Bands from Background Contaminants or Common Products

	Microns
Carbon Dioxide*	2.9, 4.3, >15
Water*	2.9, 5.8-7.0, 8-9 some interference, >12
Nitrous oxide*	4.5 (can only be seen if CO_2 is removed
Nitrogen dioxide***	6.15
Ozone**	9.5
Peroxyacetyl nitrate**	8.6
Formaldehyde**	3.6
Acetone**	8.2
Photooxidation Product Bands**** (isobutylene)	7 - 9
Acetaldehyde****	3.7, 8.9
Methyl nitrate*****	6.25, 7.75, 9.8, 11.72

* Stephens, 1958
** Glasson and Tuesday, 1970a
*** Glasson and Tuesday, 1971
**** Japar _et_ _al_., 1974
***** Tuesday, 1961

Fortunately, many compounds have more than one diagnostic peak. For example, Glasson and Tuesday (1971) have used the 6.5 micron peak to follow the formation of nitrogen dioxide. The interference caused by water vapor absorption was corrected for by monitoring the water peaks at 6.4 or 6.8 microns. However, with the six fluorocarbons that Japar _et_ _al_. (1974) studied, only three compounds had sufficient diagnostic peaks to allow long duration irradiations. Interferences from relatively inert contaminants (e.g., carbon dioxide) can be removed by using a double-beam (reference and sample cell) infrared spectrometer.

The sensitivity of the method is dependent upon the effective pathlength of the LPIR cell, the intensity of the incident infrared light, and the extinction coefficient of the diagnostic peak. The effective pathlength of the cell is limited by the reflection efficiency of the mirrors. Stephens (1958) has demonstrated that the optimum pathlength is reached when the energy reduced by reflection losses is $\frac{1}{e}$ the initial value. Glasson and Tuesday (1970a.b.c) used a 120 meter path-length to measure low concentrations of nitrogen dioxide (0.02 ppm) and hydrocarbons (>1.0 ppm). Japar et al. (1974) reported an experimental error of ±1% (2% concentration error at 50% transmittance) for fluorocarbons at 5 to 8 ppm with a LPIR cell path-length of 40 meters. Bufalini (personal communication) is able to use ppb levels of hydrocarbons with Fourier transform IR.

b. Gas Chromatographic Analysis

Since many of the compounds being studied are volatile organic chemicals (e.g., hydrocarbons), extensive use of gas chromatography has taken place. The simplest application of this technique is the direct injection of the dilute air sample. Bellar et al. (1962) described a continuous sampling system used with a dynamic smog reaction chamber. The system injected 1-cc volumes of air into a gas chromatograph with a flame ionization detector (GC-FI) and had a detection limit of 0.001 ppm for low molecular weight hydrocarbons in complex atmospheric mixtures. Direct analysis of aromatic hydrocarbons in photochemically irradiated mixtures using a 1,2,3-tris(2-cyanoethoxy) propane stationary phase with GC-FI was reported by Clemons et al. (1963). The limits of detection were as low as 0.01 to 0.1 ppm.

Several researchers have found that direct injection gas chromatographic analysis does not provide the sensitivity necessary (e.g., Altshuller et al., 1970b and Kopczynski et al., 1972) and have resorted to preconcentration of the sample before analysis. Preconcentration or trapping techniques have both advantages and disadvantages. The advantage is that the sensitivity moves into the ppb range. The disadvantages include the need for larger air samples (need larger reaction vessels) and the fact that the procedure is considerably more time consuming. The technique usually consists of trapping the compound of interest on some packing maintained in a "U" shaped cold trap. For analysis the trap is connected to a gas chromatograph and the trap is warmed to release the compound. Bellar et al. (1963) and Stephens and Burleson (1967) reported a sensitivity as low as 0.1 ppb for 100-cm^3 samples using the trapping technique with GC-FI. Jaffe and Smith (1974), using a sampling loop, reported a lower limit of detection of 0.03 ppm for acetaldehyde, total hydrocarbons, and propylene. A relative standard deviation of 10% is likely with this technique (APHA, 1972, p 132).

Atmospheric samples have also been analyzed by gas chromatography with electron capture detection (GC-EC) when the compound of interest is more sensitive or more specific to electron capture. Peroxyacetyl nitrate (PAN) is commonly analyzed with this type of detector. Kopczynski and coworkers (1972) report a sensitivity of 0.1 ppm using a preconcentration procedure with GC-EC. The detector is normally calibrated using a sample from a long-path infrared spectrometer cell which has been analyzed using reported absorptivities. The electron capture detector is also used in the study of halogenated compounds for which the detector is extremely sensitive and specific (e.g., 0.3 ppb for $BrCF_3$; Saltzman et al., 1966).

c. Colorimetric Analysis and Instrumental Methods

Colorimetric methods are commonly used to analyze for nitrogen oxides, aldehydes, and oxidants in atmospheric reactors. The following sections will discuss the analysis techniques used for each of these pollutants. These analytical procedures, because of their sensitivity, require relatively high continuous sampling rates (0.7ℓ/min or 2.3%/hr of a 64 ft^3 chamber, Dimitriades, 1967) which requires a fairly substantial initial volume.

Nitrogen Oxides

Nitric oxide (NO) and nitrogen dioxide (NO_2) are the two nitrogen oxides most frequently monitored because of their importance to the photochemical smog process. Quantitative analysis of nitrogen dioxide is provided by absorption in a mixture of sulfanilic acid, N-(1-naphthyl)-ethylenediamine dihydrochloride, and acetic acid which forms an azo dye absorbing at 550 nm (Griess-Saltzman Reaction) (APHA, 1972, p 329). A precision of 1% of the mean can be achieved in the 0.005 to 5 ppm range with careful procedure, when sampling is conducted in fritted bubblers (500 ml/min for 4 min. was used by Altshuller and Cohen, 1963). Some small interferences are caused by SO_2 (ten-fold ratio-no effect), O_3 (five-fold ratio-small interference), and PAN (15-35% response for equivalent molar concentration to NO_2).

Nitric oxide is analyzed by oxidation to nitrogen dioxide and then quantitation by the colorimetric method described above. In order to use this method, the background NO_2 must be chemically removed before the chromic oxide oxidation of NO. The range of detection is 0.005 to 5 ppm and the sensitivity is 0.01 μg/10 ml of absorbed material (APHA, 1972, p 325). Nitric oxide can also be measured by chemiluminescence with sensitivities in the low ppb (Jaffe and Smith, 1974).

Aldehydes

Aldehydes are common reaction products and reactants in photo-oxidative atmospheric reactions, and therefore are routinely analyzed in atmospheric reactors. Formaldehyde is determined by a chromotropic acid colorimetric method (APHA, 1972, p 194). A concentration of 1 ppm of formaldehyde in a 25 ℓ sample can be analyzed by this method and therefore the technique is only applicable for sizable reaction vessels. Similar high volume sampling rates (2ℓ/min) are necessary with a sodium bisulfide collection systems to determine the following minimum concentrations.

Table 33. Minimum Concentration for Sodium Bisulfide Collection Technique (APHA, 1972, p 190)

Compound	Quantitation Method	Minimum Concentration
CH_2O	Chromotropic acid	0.02 ppm
CH_3CHO	Gas chromatography	0.02 ppm
CH_3CH_2CHO	" "	0.03 ppm
$(CH_3)_2CHCHO$	" "	0.03 ppm
$CH_2 = CHCHO$	mercuric chloride-hexylresorcinol	0.01 ppm

Oxidants

The term oxidants can include ozone, nitrogen oxides, various peroxy compounds, and free radicals. The more specific methods, such as infrared and ultraviolet are not nearly as sensitive as the less discriminating redox colorimetry (Cohen et al., 1967). The rate at which the compounds react varies and allows for different methods of analysis. The most commonly used methods utilize the oxidation of KI to iodine followed by amperometric or colorimetric techniques. As with the other colorimetric methods discussed above, a relatively large sample is needed for sub-ppm analysis (150 ml/min)

(APHA, 1972, p. 348). However, if continuous monitoring is possible, very good accuracy in the 0.01-10 ppm range is possible (±5% from the mean). Interferences from NO_2 and PAN may be subtracted out and SO_2 interference can be removed by filtering the air stream through chromic acid paper absorber. However, other oxidants will react to liberate iodine so the term oxidants is normally used rather than ozone. An alternative ozone specific technique is reported by Jaffe and Smith (1974). They have used an ozone meter that relies upon the chemiluminescent reaction of ozone and ethylene and is accurate in the low ppb (Jaffe, personal communication).

d. Mass Spectrometry

Because mass spectrometers are able to qualitatively determine reactions species, they have been used to analyze atmospheric reaction mixtures. Wiebe et al. (1973) used a mass spectrometer directly connected to the reaction vessel by a capillary tube to study the importance of NO and NO_2 in scavenging CH_3O radicals. However, this direct interfacing of mass spectrometers has been infrequently used. More common is the separation of the compound of interest followed by mass spectral analysis.

Wilson et al. (1972b) used gas chromatography combined with mass spectrometry (GC-MS) in order to identify specific components of aerosols formed from pure compounds in the Battelle smog chamber. The isolated acid fraction of the aerosol was esterified with diazomethane and then injected into the GC-MS. Analysis of the mass spectrum provided convincing evidence of compound identification. Although the quantities used for analysis is not reported, the amount of material obtained is dependent upon the amount of particulate matter that is filtered. In a dynamically operated smog chamber, this should not be a limiting factor.

e. Bioassay

In studying atmospheric reactions physiological analysis is often substituted for chemical analysis because of the poor correlation between chemical analysis and biological affects (e.g., "eye irritation does not give a high linear correlation with any chemical measurement," Laity et al., 1973). The two most commonly used physiological effects are plant damage and eye irritation.

For plant damage evaluation, pinto beans, tobacco and petunias have frequently been used to assess the phytotoxicity of gases from irradiation chambers. In the plant exposure chamber used by Korth et al. (1964), the plants were uniformly exposed to the irradiated gas for 4 hours and the leaf damage was estimated on the third day (scale of 0-4). Several different responses noted included time of collapse, age of tissue affected, patterns of final injury, and microscopic changes in the progress of tissue collapse. Parameters such as soil type, nutrient content and light intensity need to be controlled with this technique (Heck et al., 1969).

An eye irritation evaluation facility consists of an opening into the reaction chamber up to which panelists can place their eye to determine the irritability of the gas. The facility used by Korth et al., (1964) consisted of glass manifolds where five of the ten panelists were exposed simultaneously. Activated charcoal respirators were used to separate odor response from eye irritation response. The degree of eye irritation was rated on a scale from 0-3. The eye irritation ports designed for the SRI smog chamber fit tightly enough that a respirator was not considered necessary (Doyle, 1970). Doyle (1970) has noted that the necessity for simultaneous and uniform exposure in assessing eye irritation requires large chamber facilities.

4. Evaluation of the Techniques

a. General

The techniques that have previously been reviewed have, for the most part, been used to study processes important to the formation of photochemical smog. However, this review is concerned with techniques that can be used to study the persistence and degradation of chemical substances in the atmosphere, and, therefore, the evaluation will be directed at this limited question. Evaluation of the techniques for predicting photochemical smog phenomena will be left for others to consider (e.g., Jaffe and Smith, 1974)

"The natural atmospheric environment is a complex, highly reactive system and its simulation requires the duplication of the photochemical and secondary dark reactions involving organic and inorganic substances" (Rose and Brand, 1960). A number of researchers have suggested that in order to reproduce such a complex system in the laboratory some of the following parameters must be controlled: temperature, humidity, purity of the air supply, concentration of the pollutants, chamber design and operation, spectral distribution and intensity of the light, rate of exchange between chamber and ambient atmosphere and reaction time (Rose and Brandt, 1960; Levy, 1973; Doyle, 1970; and Bufalini et al., 1972). The effects of these parameters will be discussed in the following section.

In atmospheric chamber studies, measurements of pollutant disappearance, product formation, visibility reduction, particulate formation, eye irritation, and plant damage are usually recorded. For the purpose of this review, the first two measurements will be considered the most relevant.

b. Factors Affecting Degradation

(i) Spectral Distribution and Intensity of Light

In a comprehensive evaluation of the factors affecting reactions in smog chambers, Jaffe and Smith (1974) compared the reaction rates using a "full" spectrum configuration (>290 nm, xenon arc lamp) to the rates using a "cut-off" spectrum (>350 nm, 3/16" Plexiglass filter with xenon arc lamp). The light intensity was kept constant for both configurations (NO_2 $K_d = 0.3\ min^{-1}$). Of the three independent variables studied (chamber material, spectrum, and surface/volume ratios), the spectral change caused the largest variation; the "cut-off" spectrum clearly slowed the reaction rate relative to the "full" spectrum.

In contrast, Stephens (1973) compared the irradiation of an ambient air sample in a borosilicate carboy with natural sunlight and blacklight fluorescent lamps (simulate sunlight fairly well in the 300-400 nm range, see Crosby, 1969b). The sunlight sample showed more reaction, but the relative reactivities of the hydrocarbons were very similar. This difference in rate may be explained by the difference in intensity (sunlight-brightest day, NO_2 half-life = 1 min; artificial light, NO_2 half-life = 1.3 min) of the difference light sources. Furthermore, Laity (1971) concluded that "the differences between blacklight lamps and natural sunlight do not dramatically influence photochemical smog formation with the systems investigated."

Kopcznski et al. (1972) determined the effect of light intensity by irradiating an ambient air sample in a plastic (Tedlar) bag with full sunlight and sunlight attenuated by 58%. They found that the ratio of losses for various hydrocarbons comparing full sunlight to 42% of full sunlight was quite different and concluded that on days of greater intensity the less reactive hydrocarbons would participate more in photochemical reactions in the atmosphere.

(ii) Concentration of Reactants

(a) Humidity: The concentration of water vapor in the atmosphere being studied appears to have a large affect on the reaction rates. Dimitriades (1967) demonstrated a considerable fluctuation in the ethylene reactivity (1.65 ppm as C in 0.50 ppm NO), as measured by rate of NO_2, oxidant, or formaldehyde formation, when the relative humidity was varied from 2.0 - 49.6% (77°F). He concluded that "the need for humidity control in chamber operations is imperative" (Dimitriades, 1967). In a study of the effect of water vapor on the photooxidation of α-pinene, Ripperton and Lillian (1971) observed that increasing the humidity decreased the net mean/time oxidant and O_3 production and net maximum condensation nuclei production, but had an insignificant effect on the average mean/time NO_2, NO and α-pinene concentrations.

Wilson and Levy (1970) have reported that the interaction between SO_2 and photochemical smog is dependent upon the water vapor concentration. In contrast, Bufalini and Altshuller (1969) found no change in the rate of oxidation of nitric oxide (with various hydrocarbons) when the water vapor was increased from 1.1 to 11 mm. of Hg. This result conflicts with the water vapor effect noted by Dimitriades (1967). Bufalini and Altshuller (1969) have postulated that the increase in hydrocarbon reactivity caused by the increase in water vapor may be due to a deactivation of active sites on the wall by the water vapor, thus enabling free radicals to have a longer lifetime. They felt that their glass chamber was either conditioned for free-radicals (from prior irradiations) or the glass system is less reactive than the smog chamber of Dimitriades (1967).

Hydrolysis reactions in the atmosphere effected by water vapor may also be important in some instances. For example, Collier (1972) found that although bis-chloromethyl ether was stable for 18 hours at 10 and 100 ppm in 70% relative humidity air, chloromethyl methyl ether hydrolyzed rapidly.

(b) NO-Hydrocarbon Concentrations: The atmospheric photooxidation of nitric oxide in the presence of hydrocarbons is the first and one of the most important reactions in the complex series that results in photochemical smog. For this reason, the reaction has received a great deal of study

(Altshuller and Cohen, 1963; Altshuller et al., 1969a; Altshuller et al., 1969b; Glasson and Tuesday, 1971; and Glasson and Tuesday, 1970b). Many studies have varied the hydrocarbon and NO concentrations in order to determine the kinetics of the reaction and the differences between studies with individual hydrocarbons in comparison to mixtures. Glasson and Tuesday (1970a) in a study of the effects of nitric oxide concentration on the photooxidation of propylene, ethylene, trans-2-butene, isobutene, and m-xylene using LPIR, found the following: (1) low concentrations of NO increase the NO photooxidation rate, (2) the rates of hydrocarbon photooxidation reach a maximum at a certain NO concentration, above or below which the HC photooxidation decreases, (3) decreased hydrocarbon concentrations decreased the rate of hydrocarbon disappearance and product formation at all NO concentrations. The initial hydrocarbon concentration has also been found to affect the NO_2 peak concentration, NO_2 dosages, oxidant dosages, peroxyacetyl nitrate formation and eye irritation (Altshuller et al., 1970a). Glasson and Tuesday (1971) have demonstrated that (a) the nitric oxide photooxidation rates observed with hydrocarbon mixtures are consistently less than the rates calculated from independent experiments with the single hydrocarbon system and (b) rates calculated from linear extrapolations of data obtained with 1.0 ppm of the individual hydrocarbons agree closely with the observed rates for the

1 ppm total HC mixtures. This result is consistent with increased NO inhibition with increase in the total hydrocarbon concentration.

(c) Other Reactants: Besides water vapor, NO_x and hydrocarbons, a number of other contaminants have been shown to have an impact on atmospheric reactions. For example, Gitchell *et al*. (1974) have demonstrated the inhibition of NO photooxidation (with hydrocarbons) by phenol, benzaldehyde, and aniline. In a study of the photochemical reactivity of benzaldehyde with NO-HC mixtures, Kuntz *et al*. (1973) reported a similar decrease in the rate of oxidant formation, hydrocarbon consumption, and NO conversion. Westberg and coworkers (1971) reported that carbon monoxide accelerates the photochemical reaction of isobutene and NO. Wilson *et al*. (1972a) demonstrated that the presence of SO_2 reduces the maximum NO_2 concentration. Also, sulfur dioxide was found to reduce the maximum oxidant obtained from 1-butene, 1-heptene, and 2,2,4-trimethylpentane, but it increased the oxidant obtained from toluene. Altshuller *et al*. (1967) have reported that hydrocarbons can be photooxidized with aliphatic aldehydes even in the absence of NO, although the rates are somewhat slower than those induced by NO.

(iii) Temperature

Bufalini and Altshuller (1963) studied the effect of temperature on the photooxidation reaction of *trans*-2-butene and 1,3,5-trimethylbenzene with nitric oxide in air. The results indicate about a two-fold decrease in conversion times for nitrogen dioxide over the 20° interval from 20° to 40°C and a corresponding increase in rates of reactions (hydrocarbons disappear almost twice as fast). The authors suggest that the results imply that the temperature changes may be dependent upon the nature of the hydrocarbon. With the trans-2-butene-nitric oxide system, the temperature change has been equated in magnitude to an increase caused by changing the light intensity from $K_d = 0.20$ to $K_d = 0.37\ \text{min}^{-1}$. Doyle (1970) has concluded that these results suggest that the temperature in a chamber should be controlled to within only a few degrees since the lack of precision of most analytical methods would make more precise temperature control useless.

(iv) Chamber Configuration, Construction Materials, and Cleaning Techniques

The importance of surface or wall reactions as they relate to "adsorption on the chamber surface, chemical reaction or decay of the gases, chemical reactions catalyzed by the chamber surface, and the formation of new components from the original precursors" has been recognized for a long time (Rose and Brandt, 1960). These processes are probably quite important when considering atmospheric reactions of low volatile materials in reaction chambers. Unfortunately, in most cases, these processes are little understood. However, in an attempt to minimize these effects researchers have varied the construction materials (made as inert as possible), the surface/volume ratio (S/V), cleaning techniques, and chamber configuration. These parameters will be briefly discussed in the following paragraphs.

The shape of the chambers seems to be more dependent upon convenience than upon any suspected effect due to various configurations. The elongated shape of the SRI smog chamber is dictated by construction costs, type of stirring pump (sonic jet-type), and the ability to accommodate a five-membered eye-irritation panel. Jaffe and Smith (1974) used a hexagonal prism shape; Laity and Maynard (1972), cylinder; and Stephens (1973), carboys. A basic consideration is that the chamber contents should be homogeneous, since inhomogeneous mixing can have an effect on the reaction rate (Donaldson and Hilst, 1972). As a result, most chambers contain some type of mixing apparatus. But even the rate of mixing may have an effect as Wilson _et_ _al_. (1971) have demonstrated for aerosol formation. It is not certain whether aerosol formation is inhibited or whether the aerosol is coated on the fan blades and chamber surfaces (Wilson _et_ _al_., 1972a).

Cleaning techniques can make substantial changes in the results obtained. An important source of contamination has been reported by Gay and Bufalini (1971) in their study of the nitrogen balance of an irradiated mixture of hydrocarbons and oxides of nitrogen. They found nitric acid as a principal surface product and believed it was formed by the hydrolysis of a nitrogen pentoxide intermediate with water vapor on the wall surfaces. Bufalini et al. (1972) suggested that "dirty" chambers may be responsible for the poor quantitative agreement between various smog chambers. They noted drastic differences (no ozone formed) in the irradiation of biacetyl in a clean borosilicate flask (rinsed with base to remove HNO_3) in comparison to results with a flask that had previously been used for biacetyl-NO_x photolysis and not cleaned. With tetramethylene (TME) irradiation, they found no reaction in the clean flask, although TME was degraded in an unclean flask (one biacetyl-NO_x-irradiation, evacuated, and then flushed with tank air). These results may also explain the differences observed between irradiation of ambient air samples in conditioned and non-conditioned plastic bags (Kopczynski et al., 1972). Conditioning was achieved by irradiating an air sample in a new bag for a full day prior to an experiment. Jaffe and Smith (1974) compared two cleaning techniques (1) purging (4.6 chamber volumes) at 110°F or (2) vacuum off-gassing (2μ pressure or less for 16 hours). The different cleaning techniques had an appreciable effect when used with stainless steel systems.

The ratio of a chamber's surface to volume (S/V) can vary considerably (0.78 to 4.91, Jaffe and Smith, 1974). The parameter is directly related to the probability of a molecule in the chamber coming in contact, and possibly reacting, with the chamber walls. In a study that varied the S/V ratios, Jaffe and Smith (1974) reported that most of the parameters that were measured (e.g., NO_2 formation rate, NO_2 maximum, ozone maximum etc.) were affected by the S/V ratio for the four materials studied (Teflon, Pyrex, aluminum, stainless steel).

The chamber construction material also appears to be an important parameter affecting atmospheric chamber results. Altshuller and Cohen (1963) attributed the difference between Mylar film and FEP film photooxidation to the difference in light transmittance. However, the inertness of the material may also be important. Laity and Maynard (1972) compared the reactivities of gasoline vapors in a glass and stainless steel chamber and found that the HC-NO_x mixtures always reacted faster in the steel chamber. Jaffe and Smith (1974) compared the effect of the use of Teflon, Pyrex, aluminum, and stainless steel as construction materials. For such reactivity manifestations as NO_2 formation rate, time to NO_2 maximum, ozone maximum, 50% propylene destruction, and NO_2 dose, the following order of reactivity is maintained: Teflon, Pyrex, aluminum, stainless steel. Pyrex and aluminum are similar in behavior for most parameters. Preliminary analysis of the data suggests that stainless steel behaves differently than the other materials (Jaffe and Smith, 1974).

c. Internal Consistency of Results

Interlaboratory comparisons of various chambers have shown poor quantitative agreement in results of irradiation of simple hydrocarbon-nitric oxide systems (Bufalini _et al_., 1972; Jaffe and Smith, 1974). In some cases even results in the same chamber have varied over the years, due to different operation, new light source, etc. (Laity _et al_., 1973). These differences could not be attributed to analytical variations (Jaffe and Smith, 1974). However, although the quantitative agreement has been rather poor, the qualitative results (ranking of reactivities relative to a standard) can be correlated fairly well as long as the experimental conditions are somewhat similar. Laity _et al_. (1973)have reported a relative standard deviation of ±10% for these relative rates, even though their chamber performance has varied so much that ten toluene standards were required in the past four years.

Hydrocarbon-NO systems have been studied the most, but only a few studies have determined the relative reactivity measured as hydrocarbon consumption. These have been summarized by Altshuller and Bufalini (1971) and are presented in Table 34. The determination of hydrocarbon reactivity in terms of nitric oxide oxidation is much more common because of the simple analytical technique involved. A comparison of two such studies in a review by Altshuller and Bufalini (1971) showed that the ranking was generally in good agreement and followed the same general order as the relative rates determined by hydrocarbon consumption. The recent, comprehensive study by Glasson and Tuesday (1970b) on hydrocarbon reactivity (measured by NO photo-oxidation) in the presence of nitric oxide also correlates well with these results.

Table 34. Ranking of Reactivities of Hydrocarbon Consumption When Photolyzed in Presence of NO under Static Conditions (see Altshuller and Bufalini, 1971 for references).

	Ranking of Hydrocarbons		
Hydrocarbon	Schuck and Doyle (1959)	Stephens and Scott (1962)	Tuesday (1963)
Tetramethylethylene	10	10	10
trans-2-Butene	6		8
cis-3-Hexene		6	
Isobutene	1.5	2	2
1,3-Butadiene	1		
Propylene	1	2	1
m-Xylene		1	
p-Xylene	0.5	0.5	
Ethylene	0.1	0.3	
Hexanes, octanes	<0.1		
Pentanes	<0.1		

d. Comparison of Laboratory Results to Behavior in the Natural Environment

Techniques which model atmospheric reactions have little utility if they are not comparable to reactions in the natural environment. This section will briefly discuss the comparability of the results for two chemical groups: (1) hydrocarbons and (2) fluorocarbons. This covers the spectrum of fairly reactive substances to almost totally inert materials.

(i) Hydrocarbons

The reactivity of hydrocarbons has been studied under a variety of laboratory conditions and considerable monitoring data is also available. Evaluating the techniques with a large number of compounds has an advantage in that the relative rates of reaction can be compared without having to resort to the very difficult task of comparing absolute rates. However, if synergistic affects are present in the mixtures, the comparison to pure compound reactivities would be extremely difficult.

Kopczynski *et al*. (1972) have irradiated with sunlight ambient air samples collected in plastic bags. They compared their data to information obtained from irradiated air samples in glass carboys (Stephens and Burleson, 1967) and relative reactivities determined on pure HC-NO_x mixtures (Heuss and Glasson, 1968). The relative reactivities, which are presented in Table 35, are in substantial agreement. Altshuller *et al*.

Table 35. Relative Rates of Percentage Loss of Hydrocarbons Averaged Over Four Hour Irradiation (Kopczynski *et al*., 1972)

	Kopczynski *et al*. (1972)		Stephens and Burleson (1967)	Heuss and Glasson (1968)
Hydrocarbon	Rel rate	Std. dev.	(4-hr irradn.)	(6-hr irradn.)
Ethane	0.06	0.09		
Propane	0.16	0.09	0.18	
n-Butane	0.27	0.13	0.37	0.11
Isobutane	0.24	0.26	0.33	
n-Pentane	0.37	0.07		
Isopentane	0.43	0.13	0.58	
2,4-Dimethylpentane	0.99	0.27		
Acetylene	0.14	0.07	0.17	
Ethylene	1.00		1.0	1.00
Propylene	2.33	0.42	2.5	1.6
1-Butene	1.62a	0.52	2.5	1.9
2-Methyl-2-butene	9.39b	1.99		2.1
Toluene	0.56	0.34		0.87
m-Xylene	1.14	0.45		1.2
o-Xylene	0.70	0.28		1.2
sec-Butylbenzene	1.25c	0.45		0.47

a Contains isobutylene.
b Averaged over first hour of irradiation.
c Contains 1,2,4-trimethylbenzene.

(1970b) also irradiated with sunlight ambient air samples in plastic bags. They were unable to detect acetylene reaction and the butanes reacted very slowly. However, the relative rates of the other paraffinic and olefinic hydrocarbons were reasonably consistent with other studies.

The studies cited above are still, in a sense, laboratory studies since static conditions were used. In comparing these results, the more dynamic nature of the open atmosphere must always be kept in mind (Kopczynski et al., 1972). However, direct comparison of ambient air monitoring data to laboratory results is extremely difficult because of varying imputs of different hydrocarbons. Nevertheless, some qualitative comparisons are possible. Stephens (1973) has noted that acetylene, ethylene, and propylene form a unique set of compounds since (1) they are derived almost exclusively from auto exhaust, (2) they are emitted in known ratios to each other, and (3) they differ quite widely in reactivity. If one assumes that the only source of the three compounds is from automobile exhaust, dilution should have no effect on the ratio; only the difference in reactivity should affect the relative concentrations. Table 36 lists the compound ratios for an air sample taken in the early morning (little photodegradation) and one taken in the late afternoon. The order of reactivity suggested by the monitoring data (ethylene>propylene) is consistent with reactivities derived under static conditions in the laboratory.

Table 36. Comparison of Acetylene, Ethylene, Propylene Ratios of Two Ambient Air Samples (derived from data in Stephens, 1973)

	Hydrocarbons in Morning Air (0735-0800 hrs.) (ppb)	Ratio C_2H_2= 1.00	Hydrocarbons in Polluted Air (1500 hours) (ppb)	Ratio	% Change in Ratio
Acetylene	78	1.00	27	1.00	-
Ethylene	64	0.82	16	0.59	27%
Propylene	14	0.18	1	0.04	78%

(ii) Fluorocarbons

Fluorocarbons are released to the environment in large quantities mainly due to their use as aerosol propellants. Three studies under laboratory conditions of the atmospheric stability of these compounds are available. Saltzman et al. (1966) examined bromotrifluoromethane and octafluorocyclobutane as potential meteorological tracers by exposing the compounds (100-250 ppb) in a plastic bag to light, ozone, water vapor, SO_2, and diluted automobile exhaust. They found no reactivity. However, these compounds are not commercially important and no ambient monitoring data are available. Japar et al. (1974) examined the photostability of fluorocarbon 11 (CCl_3F), 12 (CCl_2F_2), 22 ($CHClF_2$), 113 ($Cl_2FCCClF_2$), 114 (ClF_2CCClF_2) and 115 (ClF_2CCF_3) in a LPIR instrument in the presence of nitric oxide and an olefin. For irradiations lasting as long as eight hours, no decrease in fluorocarbon concentration was noted. Hester et al. (1973) irradiated CCl_2F_2 and CCl_3F in the absence of hydrocarbons for periods of up to two months and found no degradation.

Lovelock et al. (1973) have detected CCl_3F levels of 49.6 ppb (by volume) above the Atlantic Ocean, and Su and Goldberg (1973) report concentrations of 97 ppb and 700 ppb of CCl_3F and CCl_2F_2, respectively, in a desert area well removed from industrial sources. Based upon the above monitoring data and estimates of fluorocarbon released to the atmosphere, the above authors calculated minimum residence times of 10 (CCl_3F-Lovelock et al., 1973) to 30 years (CCl_2F_2 - Su and Goldberg, 1973). Obviously, the atmospheric stability of fluorocarbons based both on the laboratory studies and monitoring data is in good qualitative agreement.

e. General Discussion of the Advantages and Disadvantages of the Various Methods

The choice of a technique to study the atmospheric reactivity of a chemical substance has both technical and economic trade-offs. This section will discuss only technical considerations.

In general, the techniques that have been reviewed have been designed to study relatively short term (hours) phenomena. For example, although 94% of trichloroethylene reacts in 6 hours of irradiation, trichloroethylene has been characterized as "moderately reactive" (Altshuller and Bufalini, 1971). The applicability of these techniques to studies of long term processes (days, weeks, months), which are important to a determination of the persistence and fate of a chemical substance, is unknown. In addition, with the exception of the study by Crosby and Moilanen (1974), only compounds with high vapor pressures have been studied.

Chamber studies can be conducted under either dynamic or static conditions. The advantages and drawbacks of these two procedures are noted in Table 37. The size of the chamber will effect (1) the surface/volume ratio and (2) the analytical sample available and thus the concentration of reactants that must be used. The construction material can effect both the wavelength and intensity of the light source and the ability of the chamber walls to terminate radical reactions. The methods that have been used for studying atmospheric reactions will be reviewed in the following paragraphs.

Table 37. Advantages and Disadvantages of Static Vs Dynamic Procedures in Studying Atmospheric Reactions (Dimitriades, 1967; Doyle, 1970)

Dynamic		Static	
Advantage	Disadvantage	Advantage	Disadvantage
Better approximates the atmospheric situation	Instability of flow and composition introduces source of experimental error.	Simple and reliable	Less like the dynamic atmosphere
Lower effective surface/volume ratio (lower chance of wall reactions)	Need elaborate dilution, purification, and air handling system	More tractable, better precision	Higher effective surface/volume ratio
Larger analytical samples—use lower, more realistic concentrations	Limited time of irradiation	Simplifies chemical reaction system.	Smaller analytical sample-need higher concentrations
Large samples for eye irritation and plant damage studies			

Long-path infrared (LPIR) systems require a minimum of analytical development time since most compounds have a diagnostic infrared absorption. However, because of the analytical technique, the systems studied have been relatively simple (mostly HC-NO systems) and, unless one resorts to Fourier Transform IR, the concentrations of reactants are somewhat higher than what would normally be found under ambient conditions. The use of stainless steel with fluorescent lights on the inside of the chamber (Glasson and Tuesday, 1970a, b,c) seems somewhat less desirable than using more inert substances, such as Teflon (Japar et al., 1974), with the light source on the outside.

The use of plastic containers appears to be a versatile method of irradiating both artificial and natural air samples with sunlight or artificial light sources. FEP Teflon seems to be the best material because of its UV transparency. Analytical development of methods of analysis for the low concentrations that would be desirable could be quite time consuming.

Similar considerations apply to the use of glass flask reactors with the expectation that the glass reactors should be easier to rinse out for either sampling or cleaning.

Smog chambers have the advantage of larger size (lower surface/volume ratio, larger analytical sample-lower concentrations of reactants) and the potential for dynamic operation. In addition, it is easier to maintain control of humidity and temperature. However, cleaning operations are much more difficult than with plastic containers or glass reactors.

5. Cost Analysis

The equipment costs of the techniques previously reviewed can vary considerably. For example, a smog chamber may cost approximately $250,000 and a Fourier Transform LPIR, approximately $200,000, whereas a glass reactor or plastic bag irradiation unit could be assembled for well under $10,000. We find it rather unlikely that the more expensive apparatus will be used to test a compound's degradation unless the laboratory already has the equipment, but for the sake of comparison, we have included the capital costs in the per-compound calculations.

Some assumptions have been made in order to calculate the cost estimates. The labor estimates are based upon the cost of a bachelor level researcher ($12,000/year, including salary and benefits). Since most of the techniques have been designed for short-term reactions, we have assumed that the exposure time in the chambers will vary anywhere from 2 hours to 2 days. Major time commitments (and therefore costs) are involved in analytical development of techniques to determine the loss of test compound (difficult at the low concentrations required) and formation of degradation products. With LPIR, analytical development costs should be much lower. The study by Wilson _et al_. (1972b), which determined the chemical structure (using GC-MS) of the aerosols formed from a number of olefins, is perhaps typical of what would be necessary for a thorough study. In terms of cost, we have distinguished between studying the loss of test compound and identification of the breakdown products.

a. Loss of Test Compound

(i) Only One Compound Studied

	Equipment	Labor (cost) ($125/day)	Cost per Compound
LPIR	60,000 (not Fourier Transform)	3 days ($375)	60,375
Glass Flask or Plastic Bag	10,000	6 days ($750)	10,750
Smog Chamber	250,000	6 days ($750)	250,750

(ii) More Than One Compound Per Year for 5 Years

	# Compounds Per Year	Equipment	Labor/year (1 person-B.S.)	Cost per Compound
LPIR	150	60,000	28,000	250
Glass Flask or Plastic Bag	50	10,000	28,000	600
Smog Chamber	50	250,000	28,000	1,500

b. Loss of Test Compound and Isolation and Identification of Breakdown Products

(i) Only One Compound Studied

	Equipment	Labor (cost) ($125/day)	Cost per Compound
LPIR*	-----	-------	-------
Glass Flask or Plastic Bag	10,000 (buy time on GC-MS $1,000/compound)	15 days ($1900)	12,900
Smog Chamber	250,000 (buy time on GC-MS $1,000/compound)	15 days ($1900)	252,900

(ii) More Than One Compound Per Year for 5 Years

	# Compounds Per Year	Total 5 yr. Equipment	Labor/year (1 person-B.S.)	Cost per Compound
LPIR*	-----	-----	-----	-----
Glass Flask or Plastic Bag	20	10,000 and GC-MS $100,000 depreciated over 10 years total for 5 yrs = 60,000	28,000	2,000
Smog Chamber	20	250,000 and GC-MS - 50,000 total for 5 yrs = 300,000	28,000	4,400

* We find it unlikely that an intensive study like this would be done with LPIR because the breakdown products, in most cases, could not be identified by IR.

c. Summary

These cost calculations are very approximate. The possibility of having a 5 year program to test the atmospheric fate of chemicals is rather remote and, therefore, the costs are probably somewhat more than the estimates provided for the more than one compound studies. The estimates for the one compound studies are obviously inflated by the start-up costs. In addition, great fluctuations can occur due to analytical difficulties. In order to put this data into perspective, one researcher in the field has suggested a price of approximately \$3-5,000 per compound for a complete smog potential evaluation (measure NO oxidation, O_3 formation, etc. - not necessarily compound degradation and breakdown products) in a smog chamber.

B. Photochemical and Chemical Alterations in the Aqueous and Soil Environment

1. Photochemical Alterations

a. Introduction

Many chemicals that are released to the environment, especially the less volatile ones, eventually reside in the soil and water. These media do not provide as good a matrix as the atmosphere for photochemical alterations due to the possible attenuation of the incident light. In soil, only compounds that reside on the very top are susceptable to photolysis. Although water is transparent to ultraviolet light (transmits light >180 nm), the intensity falls off with increasing depth and below a few meters photolysis probably proceeds at infinitesimally small rates (Crosby, 1972a). Nevertheless, photolysis has been shown to be important to the environmental fate of chemicals which have low vapor pressures such as pentachlorophenol (Kuwakara, 1966a, b), pyrethrins (Chen and Casida, 1969), and dieldrin (Henderson and Crosby, 1968).

This section will discuss the experimental techniques used to study the photolysis of chemicals in the non-atmospheric environment. For the most part, the chemicals studied have been pesticides, and therefore the conditions that are used are attempts to simulate field conditions (Crosby, 1969b). However, when an attempt is being made to determine the photochemical reaction pathways, the experimental conditions poorly simulate the environment, but are frequently necessary in order to isolate products. Table 38 lists a considerable number of the more recent photolysis studies along with the chemicals studied and the conditions used.

Table 38. Experimental Conditions of Pesticide Photolysis

Reference	Chemical Studied	Physical State of Test Chemical	Light Source	Wavelength	Intensity	Analytic Procedures
Addison *et al.* (1974)	2 carbamates (Matacil and Landrin)	Solution - ethanol and cyclohexane	Hanovia high-pressure 1000 watt Xenon-Hg lamp with 2 Corning filters	>300 nm		TLC and GC-FI
Andelman and Suess (1971)	3,4-Benzpyrene	Absorbed on $CaCO_3$ in aqueous solution (pH 9)	White fluorescent lamps	>350 nm	0.31 mw/cm^2	Aceton extraction of $CaCO_3$ and UV determination of parent molecule
Alley *et al.* (1973) Alley *et al.* (1974)	Mirex Mirex & Kepone	Solution-cyclohexane and isooctane (0.04 M), N_2 flushed	Hanovia 450 W, medium press, Hg lamp	quartz filter		GC, preparative GC, IR, NMR, MS
Aly and El-Dib (1971) Aly and El-Dib (1972)	4 carbamates-Sevin, Baygon, Pyrolan, and Dimetilan	Aqueous solution (pH 5.0, 7.0, 9.0)	Two-15W germicidal tubes irradiated into open 9-cm petri dishes	254 nm		TLC 4-aminoantipyrine method for phenols
Archer *et al.* (1972)	Endosulfan	Thin film on borosilicate glass (0.2g on 20 x 20 x 5 dish)	GE germicidal lamps	254 nm	10^4 ergs/cm^2	TLC GL-EC
Bandal and Casida (1972)	2-*sec*-Butyl-4,6-dinitrophenol and the isopropyl carbonate	Thin film on glass and bean leaves	Sunlight	>290 nm		Ether extract TLC of radiolabelled material
Baur *et al.* (1973)	Picloram, 2,4,5-T, Dicamba/free acid and salt	Thin film on glass	F 40BL Fluorescent tubes	λmax 356 nm	7-11 x 10^3ergs/ sec/cm^2	GC
Benson et al. (1971)	Chlordane	Solution-acetone (solvent and photosensitizer) Thin file glass plate petri dish-quartz cover	Hanovia 450W, Corex filter Kimax filter Germicidal lamps Sunlight	>280 nm 254 nm >290 nm		GL-EC TLC, IR, NMR MS
Bulla and Edgerley (1968)	Aldrin, dieldrin, & endrin	Solution-water (20-25 μg/l)	One 15W germicidal lamp	254 nm		GC
Chen and Casida (1969)	Pyrethrins	Thin film-glass	275W sun lamp			TLC of radiolabelled material
Cheng *et al.* (1972)	Rotenone	Solution-oxygenated methanol benzene Thin film-plant leaves glass surfaces, and silica gel chromatoplates	Sunlight Sunlamp High press., Hg lamp (450W, Corex filter) 15W germicidal lamp	>290 nm >280 nm 254 nm		TLC, radiolabelled IR, MS
Crosby and Leitis (1973)	Trifluralin	Suspension in water (tap or deionized, 50 mg/l) varied pH Adsorption on soil from hexane solution-suspended in water Aqueous methanol	Sunlight and F40BL fluorescent, lamp through borosilicate glass	>290 nm		TLC
Crosby and Wong (1973a, 1973b)	2,4,5-T, 2,4-D p-chlorophenoxyacetic acid	Solution - 100 mg/l in water at pH 8 aerated Also used acetone and riboflavin as sensitizers	Sunlight and Six-4 ft. F40BL fluorescent UV lamps mounted in a cylindrical chamber	>290 nm 300-450 nm	390-785 μW/cm^2	GC-EC GC-FI-preparative IR, MS
Crosby and Moilanen (1973)	PCB's	Suspension in water (1-10 mg/l)	F40BL fluorescent lamp through borosilicate glass Sunlight	300-450 nm		GC-EC GC-MS
Crosby and Tang (1969a)	3-(p-chlorophenyl)-1,1-dimethylurea	Solution in water (200 mg/l)	Sunlight F40BL fluorescent lamp through borosilicate glass	>290 nm 300-450 nm		TLC
Crosby and Tang (1969b)	1-Naphthaleneacetic acid	Solution in water	Sunlight F8T5BL fluorescent lamp through borosilicate glass	>290 nm		GC, TLC IR
Crosby and Leitis (1969)	Chlorophenylacetic acids	Solution-suspension in water (100 mg/l)	360W, high press Hg lamp Sunlight	254 nm >290 nm		GC, TLC IR
Cruickshank and Jarrow (1973)	Ethylenethiourea	Adsorbed on silica gel plates Solution in water	275W sunlamp Pair of UV fluorescent lamps(one 300 nm and one 350 nm) through Pyrex Rayonet photoreactor 8-300 nm phosphor lamps 8-350 nm " "	>280 nm some 285-300 mostly >300 nm	3300 μW/cm^2 300 μW/cm^2 1900 μW/cm^2	TLC-radiolabelled material
Frost *et al.* (1972)	DDT	Absorbed on silica gel chromatoplate	Hg lamp Fluorescent lamp	254 nm 366 nm		TLC
Exner *et al.* (1973)	2,2-Dibromo-3-nitrilopropionamide	Solution in water (1.0 g/500 ml)	Sunlight Sunlamp	>290 nm		
Fishbein and Gaibel (1971)	Piperonylbutoxide	Thin film on glass	Sunlamp 275 W Hanovia 450W Hg lamp with and without filters Sunlight	peak 297 nm varied >290 nm	850 μW/cm^2	TLC GC

Table 38 (continued)

Reference	Chemical Studied	Physical State of Test Chemical	Light Source	Wavelength	Intensity	Analytic Procedures
Graham et al. (1973)	Heptachlor Epoxide	Solid in KBr disk (0.35% w/w)	Sunlight Hg lamp	>290 nm 254 nm		IR GC, MS, NMR
Gray et al. (1972)	6-Methyl-2,3-quinoxalinedithiol	Solution in benzene (30 mg/325 ml) bubbling N_2	Hanovia 450W medium press. Hg lamp-pyrex filter	>280 nm		TLC, MS, IR
Grunwell and Erickson (1973)	Parathion	Solution-aqueous THF, aqueous ethanol N_2 flushed	Rayonet photoreactor	254 nm 300 nm 350 nm		GC IR, MS
Henderson and Crosby (1968)	Dieldrin	Solution-water	Sunlight	>290 nm		GC
Henderson and Crosby (1967)	Dieldrin and Aldrin	Solution-hexane, cyclohexane, methanol	Low press. Hg lamp borosilicate filter Germicidal lamp	>280 nm 254 nm		GC, TLC
Herring et al. (1972)	Arochlor 1254	Solution- suspension (water, hexane, benzene)	Hanovia utility quartz lamp Sunlight			GC
Ivie et al. (1973)	Methazole	Solution-methanol water Thin film-glass	Germicidal lamp Sunlight	254 nm		TLC-radiolabelled compounds
Ivie and Casida (1971a)	23 Pesticides	Absorbed on silica gel chromatoplate with sensitizer	Sunlight	>290 nm		TLC-radiolabelled compounds
Ivie and Casida (1971b)	Chlorinated cyclodienes	Thin film on bean leaves, glass surfaces Aqueous chloroplast suspension	Sunlight	>290 nm		TLC-radiolabelled compounds
Jordan et al. (1964)	Phenylureas	Absorbed on filter paper	UV lamp	254 nm		UV absorption
Knowles and Sen Gupta (1969)	N'-(4-Chloro-o-tolyl)-N,N-dimethylformamidine	Absorbed on silica gel chromatoplates Solution-95% ethanol	Hg UV lamp Fluorescent UV lamp Sunlight	254 nm 320-400 nm	260 µW/cm 680 µW/cm	TLC-radiolabelled compound
Kuwahara et al. (1969) (1966a,b)	Pentachlorophenol	Solution-sodium salt in water	Sunlight	>290 nm		
Langford et al. (1973)	[illegible]-NTA complex	Solution-water	Rayonet photoreactor	350 nm		TLC
Liang and Lichtenstein (1972)	Azinphosmethyl	Solution-water Thin film-glass plate	Mercury vapor lamp Sunlight	254 nm		TLC-radiolabelled compound
Lombardo et al. (1972)	Dieldrin	Thin film-glass plate	Germicidal lamp	254 nm		GC, IR
Matsuo and Casida (1970)	Two dinitrophenolic pesticides (Dinobuton and Dinoseb)	Thin film-bean leaves	Sunlight	>290 nm		TLC-radiolabelled compound
Mazzocchi and Rao (1972)	3-(p-Chlorophenyl)- and 3-Phenyl-1,1-dimethylurea	Solution-methanol anaerobic (N_2 purged)	Low press. Hg lamp	254 nm		GC, TLC
McGuire et al. (1972)	Heptachlor	Solution-hexane, cyclohexane and acetone	Low press. Hg lamp Fluorescent lamp	254 nm 300 nm		GC, EC
Mitchell (1961)	141 Pesticides	Adsorbed on paper	Germicidal lamp	254 nm		Paper chromatography
Moilanen and Crosby (1972)	3', 4'-Dichloropropionanilide	Solution-water	Sunlight Fluorescent lamp borosilicate filter	>290 nm >300 nm		GC, TLC
Moilanen and Crosby (1974)	Bromacil	Solution-water	Sunlight Fluorescent lamp borosilicate filter	>290 nm >300 nm		GC, TLC GC-MS
Mosier et al. (1969)	DDT	Thin film-on quartz	Germicidal, low press. Hg lamp	254 nm		TLC-radiolabelled compound
Mosier and Guenzi (1973)	Picloram	Solution-water	Fluorescent lamp	300-380 nm		TLC-radiolabelled compound GC
Newsom and Woods (1973)	Dinitramine	Solution-water and methanol Adsorbed on sand	Sunlight Fluorescent lamp	>290 nm >300 nm		GC, TLC-radiolabelled compound
Nordblom and Miller (1974)	4,4'Dichlorobiphenyl	Solution-2-propanol, methanol, ethyl ether, cyclohexane, acetonitrile (degassed)	Fluorescent lamps (310 nm)			GC-EC
Pape and Zabik (1972a)	Asymmetric triazin-5(4H)-one	Solution-CCl_4, benzene, methanol, water	Fluorescent lamps (300 and 350 nm) borosilicate filter			TLC, GC, IR, UV, NMR GC-MS
Pape and Zabik (1972b)	Symmetrical triazines	Solution-methanol, water	Hg UV lamp Fluorescent lamp	254 nm 300 nm		TLC, GC IR, UV, NMR GC-MS

Table 38 (continued)

Reference	Chemical Studied	Physical State of Test Chemical	Light Source	Wavelength	Intensity	Analytic Procedures
Pape *et al.* (1970)	2-(1,3-Dioxolane-2-yl)-Phenyl-N-Methyl Carbamate	Solution-water and methanol	Fluorescent lamps (254, 300, 360 nm)			TLC, GC IR, UV, NMR
Parochetti and Hein (1973)	Trifluralin, benefin, nitralin	Sprayed on soil	Fluorescent lamps (FS-40T12) λmax = 310 nm	290-340 nm		Bioassay
Plimmer and Hummer (1969)	3-Amino-2,5-dichlorobenzoic acid	Solution-water methanol, water-sodium bisulfite	Hg lamp borosilicate filter	>280 nm		GC, TLC NMR
Plimmer and Klingebiel (1971)	2,4-Dichlorophenol	Solution-water riboflavin sensitized	Hg lamp borosilicate filter	>280 nm		GC-MS TLC
Plimmer *et al.* (1967)	Amitrole	Solution-water	High press. Hg lamp various filters	220 nm		TLC-radiolabelled compounds
Plimmer *et al.* (1970)	DDT and DDE	Solution-methanol (with or within O_2)	450W Hg lamp Corex filter Pyrex filter	 >260 nm >280 nm		GC MS
Porter (1973)	36 Dye compounds	Solution-water	Carbon arc-Pyrex filter Sunlight	>280 nm		UV
Redemann and Youngson (1968)	6-Chloropicolinic acid	Solution-water	Sunlight Hg lamp	>290 nm 254 nm		UV, IR
Rosen (1967)	N,N-dimethyl-2,2-diphenylacetamide	Solution-water	Low-press. Hg lamp Sunlight	254 nm >290 nm		TLC
Rosen and Strusz (1968)	2 urea herbicides	Solution-water	Sunlight Low press. Hg lamp	>290 nm 254 nm		TLC, GC
Rosen *et al.* (1969)	Phenyl urea herbicides	Solution-water	Sunlight	>290 nm		TLC, GC
Rosen *et al.* (1970)	Chloroanilines	Solution-water FMN sensitized	Sunlight	>290 nm		TLC
Rosen and Siewierski (1971)	Aglypt	Solution-water	Sunlight Hg lamp, Pyrex filter	>290 nm >280 nm		TLC
Rosen and Siewierski (1972)	Pyrazon	Solution-water Adsorbed on silica gel plates	Sunlight Hg lamp, Corex filter	>290 nm >260 nm		TLC, IR, MS
Ross and Crosby (1973)	Ethylenethiourea	Solution-water (with and without O_2 and sensitizers)	Sunlight Fluorescent lamp borosilicate filter	>290 nm		TLC
Ruzo *et al.* (1974)	PCB's	Solution-hexane methanol	Fluorescent lamps borosilicate filter	>285 nm		GC-MS NMR
Ruzo *et al.* (1973)	s-triazines	Solution-methanol n-butanol, water	Fluorescent lamps borosilicate filter	>285 nm		TLC
Safe and Hutzinger (1971)	PCB's	Solution-hexane, methanol	Fluorescent lamp	λmax 310 nm		TLC, GC
Slade and Smith (1967)	Diquat Paraquat	Solution-water Adsorbed on silica gel	Sunlight Hg lamp, no filter Borosilicate filter	>290 nm 254 nm >300 nm		Radiolabelled compound
Smith and Grove (1969)	Diquat	Solution-water Adsorbed on silica gel	Hg lamp with borosilicate filter Sunlight	>290 nm		TLC-radiolabelled compound
Su and Zabik (1972a)	Arylamide derivatives	Suspension-dist. & river water (pH 3.1 + 7.1) (250 ppm)	450W High press. Hg lamp (Pyrex filter)	>286 nm	2 x 10^5 ergs/cm^2/sec	TLC
Su and Zabik (1972b)	an N-methylcarbamate hydrochloride	Solution-dist. and river water (250 ppm)	450W High press. Hg lamp (Pyrex filter)	>286 nm	2 x 10^5 ergs/cm^2/sec	TLC
Tanabe *et al.* (1974)	O,O-Dimethyl S-phthalimidomethyl phosphorodithioate	Solution-diethyl ether	Hg lamp with and without Pyrex filter			TLC
Ueda *et al.* (1974)	Bioresmethrin and other pyrethroids	Adsorbed on silica gel chromatoplate Solution-water	Sunlight 275W Sunlamp			TLC-radiolabelled material bioassay
Wright and Warren (1965)	Trifluralin	Thin film-glass soil	Sunlight Hg fluorescent lamp	 360-380 nm 500-600 nm		Bioassay
Zabik *et al.* (1971)	Endrin	Solution-hexane, cyclohexane	Hg lamp with filters Vycor Corex Pyrex	 >210 nm >250 nm >280 nm		GC, MS, NMR, TLC
Zepp *et al.* (1973)	Phenylmercury compounds	Solution-water acetonitrile, benzene, and sensitizers	450W med. press Hg lamp Pyrex filter Sunlight	>290 nm		GC, colorimetric

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b. Techniques Used to Determine Photoalterations

(i) Light Sources

Perhaps the most crucial factor effecting the photoproducts and their rates of formation is the wavelength and intensity of the light source. Consideration must be given to both the light source and any media that the light passes through before irradiating the test chemical.

Using actual sunlight provides the best indication that photolysis of a chemical will occur in the environment and the use of sunlight is a common experimental technique. The test chemical or the test chemical solution is often covered by quartz, borosilicate glass, or in some cases clear, plastic wrap to reduce evaporation losses and contamination. These substances are transparent to light wavelengths greater than 180 nm, 300 nm, and 200 nm respectively (Calvert and Pitts, 1966; Crosby and Tang, 1969a). However, the inherent variation of sunlight in both wavelength distribution and intensity results in poor reproducibility, inconvenience, and lack of experimental control, and therefore many researchers have resorted to artificial sources of ultraviolet light (Crosby, 1969b).

Arc and fluorescent lamps are by far the most popular artificial light sources because they provide a rich source of UV light. The mercury arc lamp in which an electric discharge is generated in gaseous mercury vapor has become the standard UV source for most photochemical research. The arc can be operated at high, medium, and low pressure and this results in a series of sharp spectral lines (low pressure) or a fairly evenly dispersed irradiation intensity over a broad wavelength range (high pressure) as is depicted in Figure 37(Calvert and Pitts, 1966).

The higher pressure mercury lamps radiate much of the energy as heat (∿ 80%, Crosby and Li, 1969) and therefore precautions must be taken to prevent overheating of the chemical being photolyzed and to protect against glass breakage. The low pressure lamps, sometimes referred to as "germicidal lamps," are cooler and far more efficient (over 90% emitted as light), but most of their intensity is concentrated at 253.7 nm, which is far below the wavelength energies found in sunlight.

The fluorescent lamp is a well-known variation of the low-pressure mercury arc. The lamp is internally coated with a thin layer of mineral or organic phosphor. The phosphor absorbs the 254 nm radiation and then readmits the radiation by fluorescence producing a diffuse, even light at somewhat lower energy than the original arc. By varying the phosphor composition a wide spectral range can be provided. The spectral distribution of two fluorescent light sources as well as a low-pressure mercury lamp, sunlight, and the UV absorption curve of ordinary borosilicate (Pyrex) glass is depicted in Figure 38.

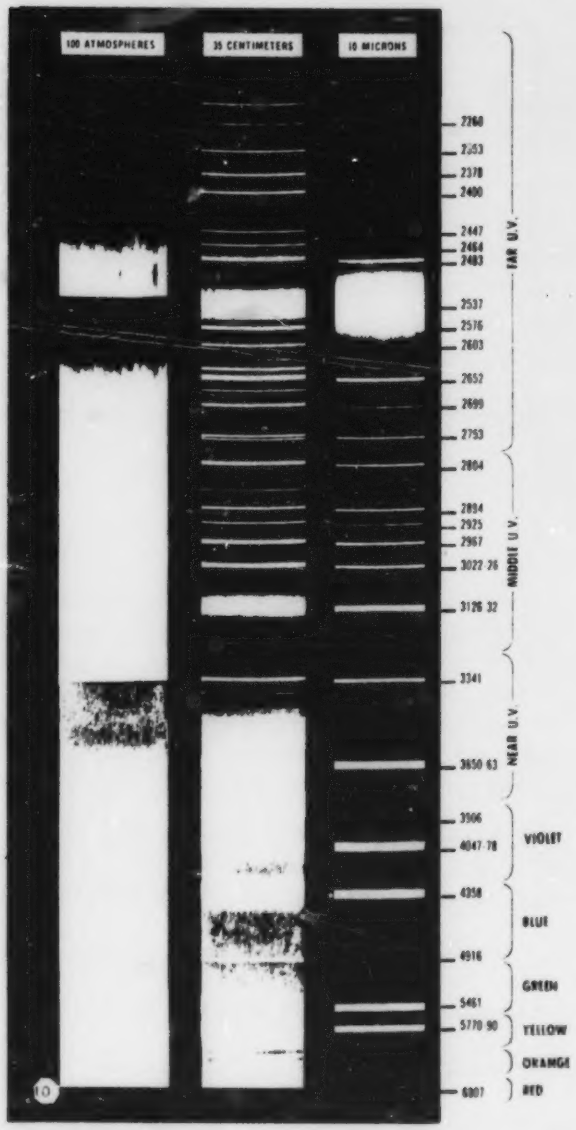

Figure 37. Emission Spectrum of the Low-Pressure (10μ), Medium Pressure (35 cm), and High Pressure (100 atm) Mercury Arcs
(Calvert and Pitts, 1966)
Courtesy of John Wiley & Sons, Inc.

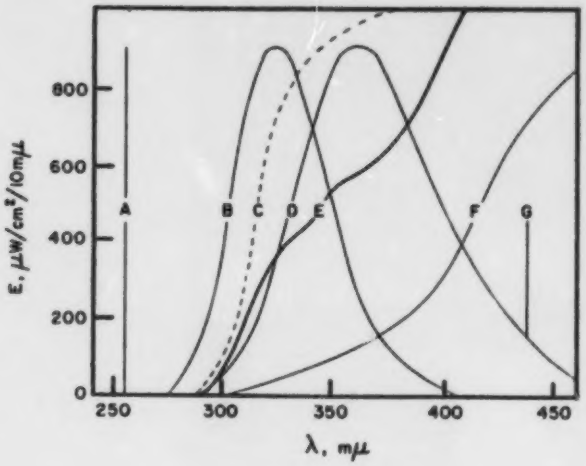

Figure 38. Spectral Distribution of a Low-Pressure Mercury Lamp (A,G); Fluorescent Sunlamp (B); Fluorescent Black-Light (D); Normal Sunlight (E); Daylight Fluorescent Lamp (F); and Transmission Spectra of Borosilicate Glass (C) (Crosby, 1969b). Reprinted with permission from Residue Reviews, 25(1) 1969. Copyright by Springer-Verlag.

As noted previously, the medium that the light passes through before striking the test chemical is also important to the intensity and wavelength of the light reaching the test chemical. Table 39 lists a variety of construction materials used to make photochemical apparatus. The transmissions indicated should be taken into account when reviewing the studies listed in Table 38.

Table 39. Approximate Wavelength Limits for Transmission of Various Materials and Water at Room Temperature (Calvert and Pitts, 1966)

Material	Thickness (mm)	Approximate λ (nm) for % Transmission Indicated		
		50%	30%	10%
Window Glass (standard)	1	316	312	307
	3	330	323	314
	10	352	342	330
Optical (white crown) Glass	1.8	327	320	309
Pyrex (Corning 774)	1	306	297	280
	2	317	309	297
	4	330	319	310
Corex D	1	278	267	250
	2	288	280	267
	4	304	292	281
Corex A	2.9	248	243	240
Vycor 790	2			> 254
Vycor 791	1	215	213	212
	2	223	217	213
	4	236	225	217
Quartz, crystal	5	185		
	10	193	192	186
Plexiglas (polymethylmethacrylate)	2.5	322	310	297
	5.0	338	325	311
	10.0	350	342	326
Water (distilled	20	188	186	185
	40	192	188	186
	80	202	194	188

(ii) Solution Photochemistry

(a) Photochemical Equipment

A frequently used technique for studying the photochemistry of a chemical is to dissolve the material in a solvent before irradiation. Solvents such as water, methanol, ethanol, hexane, cyclohexane, and benzene are often used.

The photochemical equipment used by many pesticide photochemists includes (1) Crosby's sunlight-simulating, laboratory photoreactor (Crosby and Tang, 1969a), (2) the quartz immersion well apparatus often used by Plimmer *et al*. (1967, 1970) and Rosen and Siewierski (1971, 1972) and (3) the Rayonet Photochemical Reactor sometimes used by Zabik and coworkers (Pape and Zabik, 1972a, b; Pape *et al*., 1970). Crosby's apparatus is depicted in Figure 39. The reactor is constructed of borosilicate glass and, therefore, the wavelength of light reaching the solution is no longer than 290 nm (see Curve C, Figure 38). A germicidal (low-pressure mercury) lamp may be used, but a fluorescent lamp is preferred (Crosby and Li, 1969).

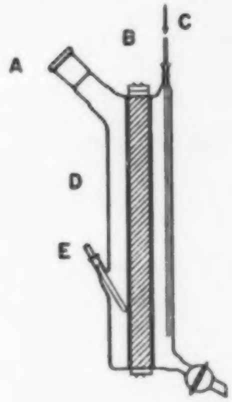

Figure 39. Sunlight-Simulating, Laboratory Photoreactor. Condenser joint (A), Lamp (B), Gas Inlet (C), Reaction Chamber (D) and Thermocouple Well (E) (Crosby and Li, 1969).

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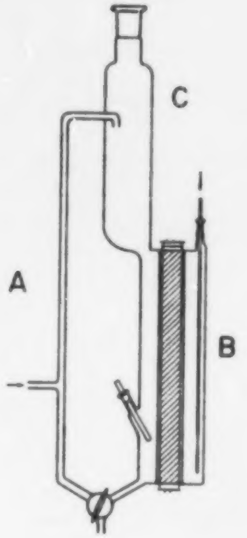
Crosby and Tang (1969b) have also used a similar device equipped for continuous extraction, in their study of 1-naphthaleneacetic acid (Figure 40). This allowed the trapping of the photosensitive intermediate photoproducts before they were degraded.

Figure 40. Photoreactor (B) Equipped with a Gas Lift (A) for Continuous Extraction (C) (Crosby and Tang, 1969b). Reprinted with permission from J. Agr. Food Chem., 17(6), 1291-1293. Copyright by the American Chemical Society.



The quartz immersion well apparatus depicted in Figure 41 is more flexible in the light energy that can be imparted to the solution. Normally a medium or high pressure lamp is placed in the immersion well. The quartz well will transmit light >180 nm but often the light is attenuated with various filter sleeves (Vycor > 210 nm; Corex, >250 nm; and Pyrex, >280 nm).

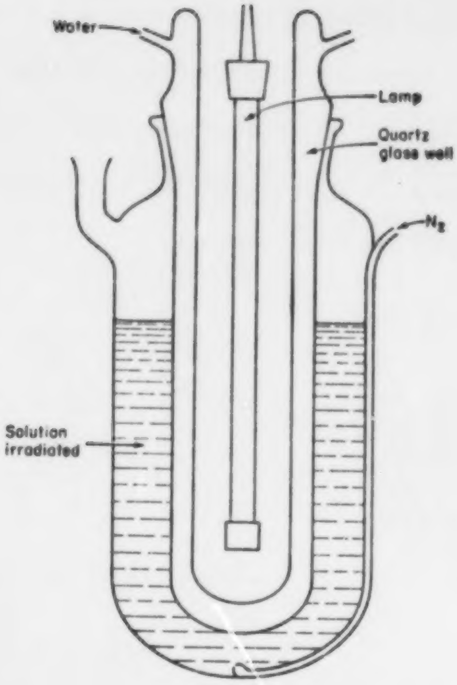


Figure 41. Quartz Immersion Well Photochemical Reactor (Kearney *et al.*, 1969)
Courtesy of Springer-Verlag

The Rayonet apparatus differs from the immersion well apparatus in that the light is provided by a bank of low-pressure mercury or fluorescent lamps which surround the reactor (λ max. of the available lamps is 254, 300, and 350 nm). Sometimes the solution is placed in a number of cylindrical tubes which are rotated (Merry-Go-Round Reactor) and construction materials may vary from quartz to Pyrex glass.

Much simpler containers such as petri dishes have also been used for solution photochemistry. For example, Crosby and Tang (1969a) used a borosilicate glass baking dish covered with a perforated clear plastic wrap (transmits light >200 nm), when irradiating an aqueous solution with sunlight Aly and El-Dib (1971) used open petri dishes when they irradiated a number of carbamate insecticides in aqueous solution with germicidal lamps (254 nm). Henderson and Crosby (1968) used an inverted, round-bottom, quartz flask for sunlight irradiation of an aqueous solution of dieldrin.

(b) Experimental Conditions

Before photolyzing a chemical in a solvent, the researcher must choose the experimental conditions to be used. Some of the parameters to be considered include (1) the light source (2) the kind of solvent, (3) a choice between aerobic or anaerobic conditions (bubble oxygen or nitrogen) and (4) a choice between sensitized (and what sensitizer) and unsensitized photolysis. The conditions that are chosen are very dependent upon the objectives of the researcher. If one is attempting to simulate the aquatic environment, water will probably be used. If one is interested in the degradation pathways, the reaction may be run in methanol or under anaerobic conditions to facilitate isolation of photolysis products.

Commonly used solvents include water, methyl or ethyl alcohol, hexane or cyclohexane, and benzene. Less frequently, solvents such as acetone (solvent and sensitizer, Benson _et al_., 1971), aqueous tetrahydrofuran (Grunwell and Erickson, 1973), 2-propanol (Nordblom and Miller, 1974), diethyl ether (Tanabe _et al_., 1974), and acetonitrile (Zepp et al., 1973) have been used. Water is used to simulate the aquatic environment while cyclohexane is used to approximate the surface of a leaf or an oil slick on water. Plimmer (1970) has used methanol as a solvent in studying the photolysis of halogenated aromatic compounds. In the compounds studied, hydrogen from the solvent ($H-CH_2OH \sim 90$ kcal) was substituted in the same position formerly occupied by the chlorine atom. In water, both hydrogen and hydroxyl substitution occurred but at a slower rate. Thus, because the work-up of the methanol solution is easier, methanol has frequently been substituted for water.

The solubility of some of the test chemicals in water precludes solution photolysis. For example, Crosby and Moilanen (1973) photolyzed a suspension of 1 or 10 mg/l of chlorinated biphenyl in water. Andelman and Suess (1971) instead of photolyzing a suspension, absorbed 3,4-benzpyrene on calcium carbonate and irradiated an aqueous suspension of the calcium carbonate.

The light used either does or does not attempt to simulate sunlight. High energy light is used to see if a compound reacts at all photochemically. In some cases, it even provides photoproducts observed in the natural environment (e.g., dieldrin forms the same photoproducts in sunlight and upon irradiation with high energy UV light).

Less energetic light is used in an attempt to more closely consider environmental processes. Low energy artificial light is frequently used with high concentrations (0.1 to 1.0 g/l) of the test chemical in order to isolate and identify the photoproducts. In many cases, these products and the rate of formation are compared with a sunlight photolysis at lower concentration (e.g., Crosby and Leitus, 1973).

The intensity of the light used has a considerable effect upon the photolysis reaction rate and, therefore, its measurement is necessary in order to compare experimental results. However, in many cases, only the light source is reported. When the intensity is determined, it usually is done with a physical or chemical actinometer (See Calvert and Pitts, 1966).

In many cases, the compound under study does not directly absorb ultraviolet light available from sunlight. However, in the environment, the chemical may come in contact with a compound that absorbs light efficiently and could donate its energy to the chemical under study (see Lykken, 1972). Therefore, many researchers have added photosensitizers to the photolysis solution. Both natural (e.g., riboflavin-5'-phosphate sodium (FMN)) (Rosen et al., 1970) and artificial (e.g., acetone, Crosby and Wong, 1973b and benzophenone, Rosen and

Siewierski, 1970), sensitizers have been used. When using benzophenone, a hydrogen donor solvent such as cyclohexane should not be used because it leads to the rapid destruction of the sensitizer (Rosen and Siewierski, 1970).

Most places in the environment that receive irradiation from the sun are aerobic and, therefore, photooxidation is often an important environmental process (Crosby, 1972b). In recognition of the importance of photooxidation, solvent photolysis is usually carried out in the presence of oxygen. However, the products formed in the presence of oxygen may be extremely complex and as a result anaerobic conditions are sometimes used to provide insight into the product intermediates as well as the mechanism (e.g., Plimmer _et al_., 1970). In some cases, oxygen must be excluded in order to isolate intermediates that are rapidly oxidized in the presence of oxygen. For example, Crosby and Tutass (1966) found that 1,2,4-benzenetriol, a major photoproduct of 2,4-D, could only be isolated by adding excess sodium bisulfite to the aqueous photolysis solution before photolysis in order to inhibit oxidation.

(iii) Adsorbed or Thin Film Photolysis

A considerable number of chemicals, mostly pesticides, have also been photolyzed as thin films on borosilicate glass, quartz, or bean leaves or in an adsorbed form on paper, silica gel, and calcium carbonate. Perhaps the first use of this technique was the work of Mitchell (1961). He photolyzed 141 pesticides that were adsorbed on 8" x 8" filter papers with a germicidal lamp (254 nm). The filter papers were then developed in a chromatographic tank to determine if the chemical had reacted photochemically. A modern variation of the technique is the use of silica gel chromatoplates (Cheng et al., 1972; Cruckshank and Jarrow, 1973; Ivie and Casida, 1971a; Knowles and SenGupta, 1969; Rosen and Siewierski, 1972; Slade and Smith, 1967; Smith and Grove, 1969; and Ueda et al., 1974). The silica gel technique has been used both with and without photosensitizers (Ivie and Casida, 1971a). The adsorption of the chemical on silica gel may have a drastic effect on the absorption spectra of the compound and thus effect the amount of direct irradiation adsorbed by the compound and the photoproducts that result (Plimmer, 1972b). Other surfaces that have been used include sand (Newsom and Woods, 1973), soil (Parochetti and Hein, 1973; Wright and Warren, 1965), and calcium carbonate (Andelman and Suess, 1971). Both sunlight and artificial light has been used in the photolysis of adsorbed pesticides.

Another technique that has frequently been used with pesticides is solid state photolysis of thin films of the compound on glass (Archer et al., 1972; Bandal and Casida, 1972; Baur et al., 1973; Benson et al., 1971; Chen and Casida, 1969; Cheng et al., 1972; Fishbein and Geibel, 1971; Ivie et al., 1973; Ivie and Casida, 1971b; Liang and Lichtenstein, 1972;

Lombardo et al., 1972; Wright and Warren, 1965), quartz (Mosier et al., 1969) and bean leaves (Bandal and Casida, 1972; Cheng et al., 1972; Ivie and Casida, 1971b; and Matsuo and Casida, 1970). Normally the chemical is dissolved in a solvent for application to the surface and then the solvent is evaporated. The possibility of intermolecular reactions under these conditions is probably quite high.

An interesting and novel way of photolyzing chemicals in the solid state was reported by Graham et al. (1973). They studied the photolysis of heptachlor epoxide in a KBr disk (0.5% w/w). Both artificial light (254 nm) and sunlight were used (same results) and the reaction was followed by infrared absorption spectra.

2. Techniques Used to Determine Chemical Alteration

a. Introduction

A chemical placed in the environment may undergo other chemical reactions besides photochemical alterations. The exposure of a chemical to water, soil, air, and inorganic and organic matter may catalyse many chemical reactions, oxidation and hydrolysis being perhaps the most common. The few studies of these processes that are available have been reviewed by Rosen (1972a) and Crosby (1969a). This section will very briefly review some of the techniques that have been used.

b. Techniques Used to Study Chemical Alterations

In almost all instances, the techniques used to study biodegradation of a compound automatically take into account chemical alterations.

However, many researchers in studying the degradation of a chemical in simulated environmental conditions have distinguished between chemical and biological degradation. This is usually done by removing the possibility of biodegradation. For example, Eichelberger and Lichtenberg (1971), in a study of the degradation of 21 pesticides in river water, distinguished the chemical alterations by using distilled water. Studies of the degradation of pesticides in soil usually include a control sample that has been sterilized (by autoclaving or treatment with ethylene oxide or potassium azide, or by γ irradiation). Kaufman _et al_., 1968) found that autoclave treatment was the most effective for retarding the biodegradation of amitrole in silty clay loam (see Figure 42).

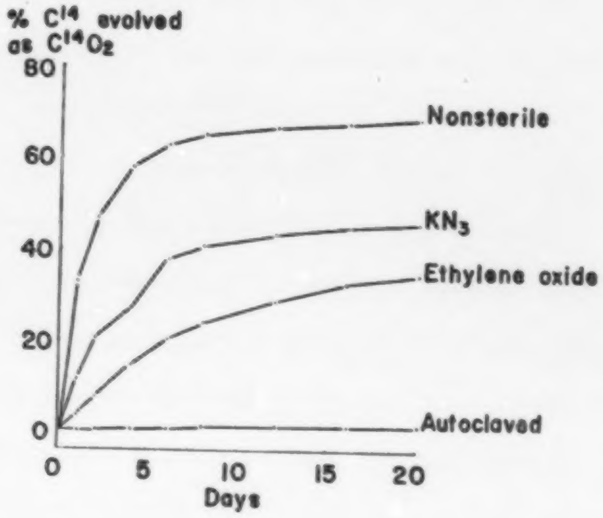


Figure 42. Effect of Soil Sterilization on Amitrole Degradation in Hagerstown Silty Clay Loam (Kaufman _et al_., 1968) Courtesy of Weed Science Society of America, publication of Weed Science.

In a modification of the sterile soil technique, Hance (1967) incubated at 107°, 95°, and 85°C a number of herbicides in an aqueous slurry with two soils (silty loam, 22.6% clay, 3.45% organic carbon, pH 6.2 in 1:5 aqueous suspension; calcareous silty loam, 33.6% clay, 3.09% organic carbon, pH 7.5 in 1:5 aqueous suspension). The elevated temperatures were used to eliminate the possibility of biological action and also to accelerate any processes that were occurring in order to reduce the length of the experiments.

Conventional studies of the hydrolysis rate of various pesticides in water have been reported by Gomaa and Faust (1972); Faust and Gomaa (1972); Aly and El-Dib (1972) and Exner _et al_. (1973). The experimental procedure consists of exposing the pesticide (organophosphorus compounds, carbamates, etc.) to an aqueous solution buffered to various pH levels (both acidic and basic) and then analyzing for hydrolysis products. Also, the effect of different temperatures is sometimes determined.

Plimmer _et al_.(1967) in a study of the degradation of amitrole found similarities between the mode of breakdown in hydroxyl-radical systems (Fenton's reagent) and soil. Fenton's reagent consists of a 0.2M solution of ferrous sulfate in 0.1M sulfuric acid (60 ml) and 1% hydrogen peroxide (100 ml). The system might be used to provide some insight into chemical degradative mechanisms in soil.

3. Analytical Procedures

a. Isolation and Detection of Degradation Products

As Table 41 shows, a variety of analytical techniques have been used with studies of the photochemical degradation of chemicals. These detection, isolation, and identification techniques vary with the particular compound and reaction conditions. Thin-layer chromatograph (TLC) appears to be the most commonly used technique for isolation despite the frequent difficulty of overlapping bands (Crosby, 1969b). The method is an obvious choice when the photolysis is carried out on silica gel chromatoplates. The combination of the two techniques (photolysis on chromatoplates and TLC work-up) provides an extremely fast technique for determining photoreactivity. For example, Ivie and Casida (1971a) screened the photosensitizing ability of 175 unlabelled pesticides against each of six C^{14}-labelled insecticide chemicals using the combination of silica gel chromatoplate photolysis - TLC. A similar technique, only using paper chromatograph, allowed Mitchell (1961) to screen the photoreactivity of 141 pesticides. The technique used to detect the spots on the chromatogram varies for each chemical but may include (1) exposure to various light sources (254 or 366 nm), (2) exposure to chromogenic agents (Mitchell, 1961) and, or (3) use of radiotracer techniques when radiolabelled matter is available for testing. When small amounts or concentrations are being used, radioanalysis is most desirable because of the low sensitivity (nanogram levels) that can be achieved (Casida, 1969). The combination of TLC with radiotracer assay is frequently used by pesticide photochemists (see Table 41) perhaps because of the availability of the radiolabelled material.

Gas chromatography (GC) has also been found to be satisfactory for the isolation and detection of volatile, thermally stable, compounds formed in photochemical reactions (see Table 41). In some cases, GC is used to quantitate compounds isolated by TLC. The sensitivity is dependent upon the detector which is in turn dependent upon the chemical being studied [minimum detectable quantity can vary from 10^{-7}g (thermal conductivity) to 10^{-13} (electron capture)(Karasek and Laub, 1974)].

Other techniques that have been used for isolation and/or quantification include (1) UV absorption (Andelman and Suess, 1971; Porter, 1973; Redemann and Youngson, 1968), (2) colorimetric analysis (Aly and El-Dib, 1971, 1972; Zepp, 1973); (3) infrared analysis (Graham _et al_., 1973); (4) polarographic analysis (Exner _et al_., 1973) and (5) bioassay (Parochetti and Hein, 1973; Ueda, 1974; Wright and Warren, 1965). These methods have the advantage of being relatively fast and inexpensive to run, but are usually only good for analysis of the parent compound and one or two degradation products.

b. Identification of Degradation Products

Although radiotracer analysis combined with chromatographic separation provides extensive insight into the degradation rates and numbers of products that might occur, the technique provides little help with structure identification (Crosby, 1969b). Gas chromatography has similar problems. In many cases, identification is only possible by cochromatography of an authentic compound from synthesis (Casida, 1969). When the degradation product is not an obvious one, physical tools, such as infrared spectroscopy, mass spectrometry, and nuclear magnetic resonance (NMR) spectrometry, are used. These techniques require the isolation of the pure compound in fairly large amounts (μg-mg), especially for NMR. However, with photochemical and chemical degradation studies, it is often possible to run preparative reactions in order to generate enough material for identification. For example, Crosby and coworkers (Crosby and Leitis, 1973; Crosby and Hammadmad, 1971) have used solutions concentrations of 50 mg/l to 1 g/l. In fact, many laboratory studies are undertaken in model systems, that do not reflect actual environment conditions, in order to facilitate the isolation and identification of products that might be formed in the environment (Rosen, 1972b).

4. Evaluation of the Techniques

a. General

The laboratory techniques which have been previously described are oriented at understanding chemical and photochemical alterations of chemicals in the environment. Although the importance of these nonmetabolic processes to degradation of contaminants in the atmosphere has been well documented, the magnitude of these processes in soil and water media relative to biological processes is for the most part unknown. (e.g., pesticide photolysis - Rabson and Plimmer, 1973). This is undoubtedly due to the fact that both metabolic and nonmetabolic processes usually only represent normal chemical transformation (Crosby, 1969a) and, therefore, the assignment of a reaction in the soil and aquatic environment to either a metabolic or nonmetabolic process is most difficult. This appears to be particularly true with photooxidations where the photodegradation products often prove to be structurally identical with products of oxidative degradation in living organisms (Crosby, 1972b). Thus, these nonmetabolic processes have been demonstrated to be important in only a few cases where an unusual and environmentally stable alteration product is formed or where attenuation of some parameter, such as light intensity or pH, has altered the rate of degradation under natural conditions. This is not to say that these processes are not important; just that their magnitude of importance is difficult to demonstrate.

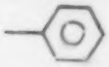
The chemical and photochemical alterations of a large number of compounds, mostly pesticides, have been studied in model systems in order to identify products that might be formed in the environment and to determine the rate of the degradation process. This section will discuss the factors affecting results obtained in model systems and consider the extrapolation of these model results to processes in the actual environment.

b. Factors Affecting Chemical and Photochemical Degradation

(i) Light Wavelength

As noted earlier the wavelength of the light source can have considerable affect upon the reaction that occurs. Many researchers have used a variety of light sources including low pressure mercury lamps (254 nm), >290 nm artificial light, and sunlight. Sometimes the results are the same (e.g., Cheng *et al.*, 1972; Benson *et al.*, 1971; Chen and Casida, 1969; Henderson and Crosby, 1968), but in many cases drastic differences are noted. For example, Henderson and Crosby (1967) reported that the dechlorination of dieldrin was wavelength dependent. Rosen (1967) detected five photoproducts of diphenamid using 254 nm light, while in sunlight the compound was stable. Moilanen and Crosby (1972) studied the photodecomposition of bromacil with sunlight. Although it had been reported that a 1 ppm aqueous solution of bromacil was completely decomposed in 10 minutes with 254 nm light, Moilanen and Crosby (1974) detected little photodecomposition using sunlight wavelengths. Liang and Lichtenstein (1972) found rapid decomposition of azinphosmethyl in water using 254 nm light, but no decomposition using yellow (589 nm) or red (653 nm) light. Redemann and Youngson (1968) suggested that "a change of light sources can result in a change in relative photolysis rates of parent compound and intermediate products". They photolyzed 6-chloropicolinic acid with sunlight and a mercury arc lamp (254 nm) and detected 6-hydroxypicolinic acid as an intermediate with 254 nm light, but did not detect the compound with sunlight. This result was attributed to the greater absorptivity and thus possible photochemical reactivity of 6-hydroxypicolinic acid in the wavelength range available from sunlight.

(ii) Reaction Media

The reaction media used in a photochemical experiment can also affect the results. Solution photolysis will reduce the possibility of intramolecular reaction, but many solvents, especially H-donor solvents, may react with the chemical being studied. Photolysis of halogenated aromatic compounds in a H-donor solvent, such as methanol, hexane or cyclohexane, results in hydrogen substitution at the halogen position. The possibility that this represents what occurs in oil slicks (C_8-C_{22} fatty acids, Crosby, 1972a) on the surface of water or on the surface of a leaf (Crosby, 1969b) seems feasible but has never been proven. In water, both hydrogen and hydroxyl substitution may occur at slow rates (Plimmer, 1970; Crosby, 1972a). The mechanism involved is quite important because solvents, such as methanol, are often used in place of water due to the solubility properties of the compound being photolyzed (e.g., Plimmer et al., 1973). Both chlorine dissociation of the excited molecule to a free radical (Plimmer, 1970) and nucleophilic displacement of the chlorine with a solvent or substrate molecule (Crosby et al., 1972; Crosby, 1972a) have been postulated. The free radical mechanism is theoretically possible in methanol ($H-CH_2OH$ = 92 kcal; H  = 104 kcal/mole), but in water (H-OH = 118 kcal) the energetics make the reaction by a free radical mechanism highly unlikely. Recently, Nordblom and Miller (1974) have demonstrated that a free radical mechanism does not seem feasible even with some H-donor solvents (cyclohexane, ethyl ether). Thus, extrapolations of methanol results to reactions in water should be done with some caution.

Adsorption of the chemical on silica gel or formation of a thin film on glass or leaves may affect the adsorption spectra of the chemical and thus affect the ability to directly derive energy from the incident light. Plimmer (1972b) has noted quite sizable λmax shifts for compounds adsorbed on silica gel. Rosen (1972b) reported completely different results for a thin film on glass photolysis of a synthetic pyrethroid as compared to photolysis on silica gel. Whether this can be explained by a λmax shift is unknown. Rosen (1972b) has concluded that "if we cannot, in some cases, extend our results from silica gel to glass, extension to soil and leaf surfaces may be even more risky." For example, Parochetti and Hein (1973) noted that trifluralin was reported to photodecompose on glass surfaces, in organic solvents, in aqueous solutions (Crosby and Leitis, 1973), and as a vapor, but their results provide no positive evidence of photodecomposition on a soil surface.

(iii) Sensitizers

In cases where direct irradiation results in a relatively slow reaction or no reaction at all, sensitizers are added to determine if the compound can react photochemically by indirect excitation. Since natural sensitizers, such as riboflavin and chlorophyll, are available in the environment, this process is possible in nature. The sensitized photodecomposition and the photosensitizer activity of a number of pesticides has been reviewed by Ivie and Casida (1971a, b) and Lykken (1972).

The effectiveness of the sensitizer is dependent upon, among other things, the wavelength at which it absorbs light, the efficiencies of the conversion from its singlet to triplet state, the efficiency with which it transfers energy to the acceptor molecular, and its concentration (see Turro, 1967). Therefore, the choice of sensitizer can have considerable impact on the rate of reaction. For example, rotenone is 100 times more effective as a sensitizer of the photoisomerization of dieldrin than is benzophenone (Lykken, 1972).

A typical study which employed the use of sensitizers, is the photolysis of ethylenethiourea (ETU) (Ross and Crosby, 1973). Photolysis of ETU (λmax = 240 nm) in deionized water revealed no loss, while in the presence of acetone or riboflavin, 95% of a .064 ppm aqueous solution was lost in 4 hours.

(iv) Hydrogen Ion Concentration

The pH of the reaction solution can have a considerable effect on the photochemical or chemical process that takes place. For example, Lanford et al. (1973) found that the quantum yield decreased linearly with increasing pH over the range 2-12 in the photodecomposition of copper complexes of NTA irradiated with 350 nm light (Rayonet unit). Crosby and Leitis (1973) reported that the principal product from photolysis of trifluralin was completely different under acidic vs basic conditions (see Figure 43).

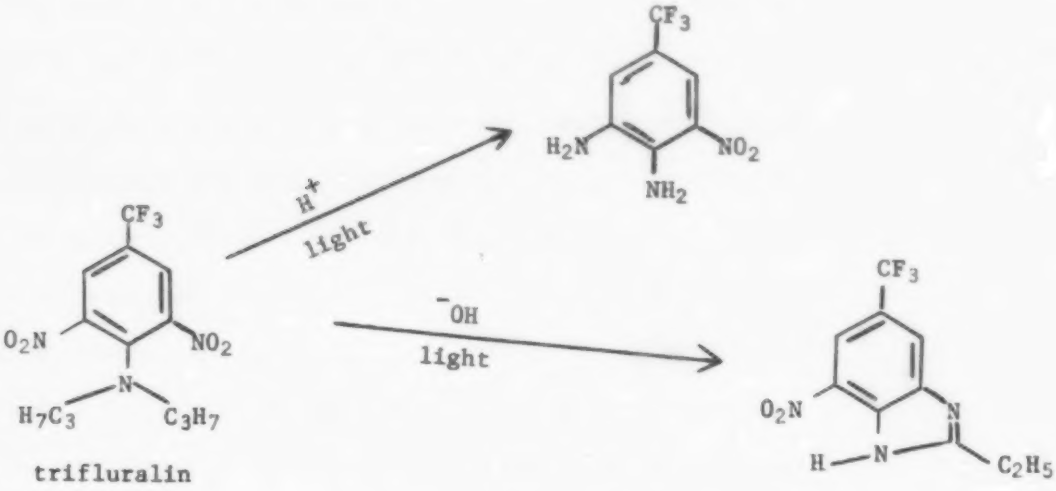

CF3
H2N
NO2
NH2
H+
light
CF3
O2N
NO2
N
H7C3
C3H7
trifluralin
-OH
light
CF3
O2N
N
H—N
C2H5

Figure 43. Basic and Acidic Photolysis of Trifluralin (Crosby and Leitis, 1973)

Crosby and Wong (1973b) also noted that the rate of 2,4,5-T photolysis was somewhat more rapid at pH8 than at pH3.

The hydrogen ion concentration has been shown to have a drastic effect on the rate of hydrolysis of carbamates, organophosphorus compounds, amides, etc. Aly and El-Dib (1972) reported that two carbamate insecticides (Sevin, baygon) were rapidly hydrolyzed under neutral to alkaline conditions. Faust and Gomaa (1972) studied the chemical hydrolysis of organic phosphorus pesticides. They noted that the basic hydrolysis occurs in a S_n2 fashion by nucleophilic substitution in which ^-OH attacks the P and substitutes for a R-O group (breaks P-O bond). In contrast, acid conditions cause the R-O bond to break (see Figure 44). In Faust and Gomaa's (1972) extensive review, they demonstrate that the hydrolytic rate is pH dependent as well as dependent upon the nature of substituents. Similar correlation between pH and hydrolysis was found for azinphosmethyl by Liang and Lichtenstein (1972). The rates of hydrolysis can fluctuate greatly. For example, Zepp et al. (1974) reported that the hydrolysis half-life (25°C) of the butoxyethyl ester of 2,4-D was 9 hours at pH 8 compared to one year at pH 5.

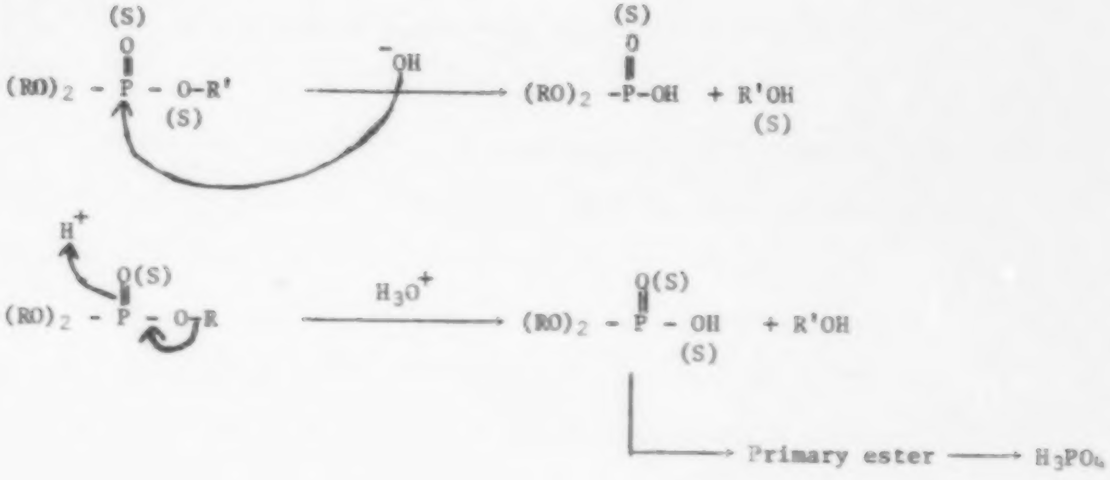


Figure 44. Acid and Base Catalyzed Hydrolysis of Organophosphorus Pesticides (Faust and Gomaa, 1972)

(v) Other Factors

Chen and Casida (1969) have suggested that, besides the factors noted previously, the purity of the irradiated materials and the methods of determining the degradation products may have a considerable affect on the results that are reported. Furthermore, Baur _et al_. (1973) have shown that concentration may be important in their film photolysis.

c. Extrapolation of Laboratory Results to Field Conditions

The ultimate evaluation of a technique used to study chemical or photochemical alterations in the environment is a comparison of results obtained in the laboratory with actual environmental results. The number of cases where indisputable field results are available is rather limited. Many studies of pesticide residues in the environment have been carried out, but provisions have not been made for volatilization nor have nonmetabolic reactions been distinguished from metabolic reactions. However, there are two examples involving photochemical reactions which are relatively unambiguous - dieldrin and pentachlorophenol. In addition, information on the photolysis of PCB's has been reviewed and compared to environmental monitoring data.

(i) Photolysis of Dieldrin to Photodieldrin

Roburn (1963) reported the presence of an unknown contaminant on samples of herbage sprayed with dieldrin. The substance was later identified as photodieldrin (see Figure 45 by Parsons and Moore (1966), Robinson et al. (1966), and Rosen et al. (1966). In a monitoring survey, Robinson and

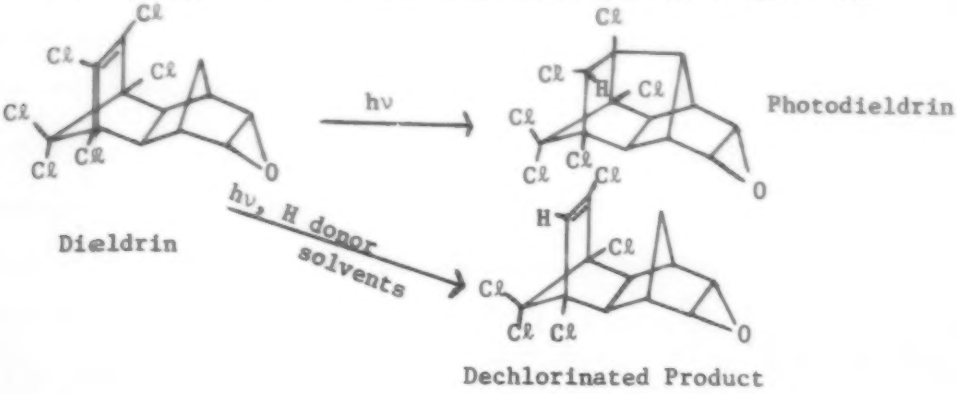

Cℓ
Cℓ
Cℓ
Cℓ
Cℓ
Cℓ
O
Dieldrin
hν
Cℓ
Cℓ
H
Cℓ
Cℓ
Cℓ
Cℓ
O
Photodieldrin
hν, H donor solvents
H
Cℓ
Cℓ
Cℓ
Cℓ
Cℓ
O
Dechlorinated Product

Figure 45. Photolysis of Dieldrin

coworkers (1966) found that photodieldrin occurred in very small amounts (16:1 to 1000:1 compared to dieldrin) in the environment (e.g., cooked meals, human fat, shag eggs). Photodieldrin was also detected by Henderson and Crosby (1967) on corn leaves treated with dieldrin and exposed to sunlight.

The photoproduct has been produced by 254 nm light irradiation of a thin film deposited on filter paper (Robinson, et al., 1966) or glass plates (Rosen et al., 1966) and by sunlight irradiation of an aqueous solution (Henderson and Crosby, 1968), a thin film deposit on a glass plate (Rosen, et al., 1966), or on various media with a sensitizer (Ivie and Casida, 1971b). Rosen and Carey (1968) formed photodieldrin in 75% yield by benzophenone-sensitized photolysis of dieldrin in benzene with 268-356 nm light for 21 hours. Irradiation of dieldrin in hexane with 254 nm resulted in the formation of a dechlorination product that is not detected in the field and is not formed at light wavelengths greater than 260 nm.

That dieldrin is photoaltered in the environment is, of itself, somewhat unusual. In hexane solution, dieldrin has an absorption band appearing at 260 nm and reaching a maximum at 215 nm. Since the compound is transparent to wavelengths greater than 260 nm, it should not be directly excited by sunlight because wavelengths below 285 nm do not reach the earth's surface (Crosby, 1969b). Nevertheless, dieldrin in distilled water, adsorbed on paper, or in a thin film on glass is converted to photodieldrin by sunlight. Two explanations are possible. Rosen (1971) has cited a report which suggests that the solar flux reaching the earth between 200-285 nm is significant, although quite small. Rosen suggests that this energy is concentrated in the 200-220 nm range since the absorption coefficient of ozone drops off sharply at 220 nm and therefore "it is not surprising that they [cyclodiene insecticides] undergo appreciable photolysis in sunlight " (Rosen, 1971).

Another possibility is that the wavelength of absorption is shifted in different media making direct excitation possible (for examples, see Plimmer, 1972b). Unfortunately, no one has measured the ultraviolet absorption of dieldrin in water. This possibility of wavelength shift is somewhat supported by the fact that dieldrin in hexane does not form the dechlorinated product, which is favored in hydrogen donor solvent at wavelengths less than 260 nm (Henderson and Crosby, 1967).

Another explanation is that dieldrin is photosensitized in the natural environment (Rosen and Carey, 1968; Ivie and Casida, 1971b), but this would not explain the photodecomposition of dieldrin in distilled water (Henderson and Crosby, 1968).

A complicating factor with photodieldrin is that it can be formed microbially (Matsumura and Boush, 1967). One would expect that the photodieldrin formed on the surfaces of leaves is probably the result of photolysis, but the rate determining process in soil and water is unknown.

(ii) Photodegradation of the Sodium Salt of Pentachlorophenol

The sodium salt of pentachlorophenol (PCP-Na) is used in Japan as a control of barnyard grass in rice paddys. This substance is highly toxic to fish, but the toxicological activity in the water disappears several days after treatment. However, the fish toxicity can be prolonged by covering the surface of the field water with sheets (Munakata and Kuwahara, 1969) which is good evidence of the photodegradation of PCP-Na by sunlight. In order to isolate and identify the degradation products, Munakata and Kuwahara (1969) irradiated with sunlight 1 Kg of PCP-Na in 50ℓ of water. In ten days the solution turned purple and the PCP-Na content decreased by 50 percent, which is qualitatively in good agreement with field data. Unfortunately, a quantitative comparison between field and laboratory results of PCP-Na loss and degradation product formation is not available.

(iii) Photolysis of Polychlorinated Biphenyls

PCB's provide a good example of the problems that arise in attempting to extrapolate laboratory photolysis results to behavior in the environment. Safe and Hutzinger (1971) found that 2,4,6,2',4',6'-hexachlorobiphenyl was readily dechlorinated to lower chlorinated isomers when irradiated with λmax 310 nm light in hexane. Upon irradiating 2,2',5,5'-tetrachlorobiphenyl under similar conditions (the solution is degassed) only 34%

of the starting material remained after 41 hours (Hutzinger et al., 1972). The hexa- and octachlorobiphenyl were extremely photochemically labile under those conditions, much more so than the tetra isomer. Photolysis in hydroxylic solvents at pH 9 yielded hydroxyl and carboxyl products (Hutzinger et al., 1972). Ruzo et al. (1972) reported the stepwise reductive dechlorination of 3,4,3',4'-tetrachlorobiphenyl and 4,4'-dichlorobiphenyl in hexane under 300 nm irradiation. Herring et al. (1972) irradiated Arochlor 1254 in hexane, water and benzene (all the solutions contain 1% acetone) with 254 nm light and sunlight. Gas chromatographic analysis showed that some of the peaks increased (due to reductive dechlorination of higher isomers) and the degradation was fastest in hexane, then water, and slowest in benzene. Crosby and Moilanen (1973) irradiated a variety of chlorobiphenyls (di, tri, tetra) in aqueous suspension with the sunlight-simulating, laboratory photoreactor. They isolated both reduced and hydroxylated product. These authors concluded that "photodecomposition of the lower chlorinated isomers in both polar and nonpolar solvents reveals the operation of environmental mechanisms which could effectively degrade these widespread contaminants." They further suggest that "the lack of lower chlorinated biphenyls from most environmental samples is suggestive of an important role of photodegradation in the environmental fate of PCB's" (Crosby and Moilanen, 1973). Nordblom and Miller (1974) photolyzed 4,4'-dichlorobiphenyl in degassed 2-propanol and methanol with 310 nm light and formed 4-chlorobiphenyl. In ethyl ether, cyclohexane, or acetonitrile no photoreduction occurred and the authors suggested that this data does not support a homolytic free radical mechanism. Ruzo et al. (1974) studied the photolysis rates of six tetrachlorobiphenyl isomers in hexane and methanol solution. The rate of reaction does not follow the magnitude of the molar extinction coefficient. The order in which the chloride atoms are cleaved is ortho>meta>> para. In summary, the chlorinated biphenyls seem to react photochemically with >290 nm light and the higher isomers would seem to be more labile than the lower ones, although Crosby and Moilanen (1973) have argued that the lack of lower isomers in the environment would suggest a photochemical process.

Nisbet and Sarofim (Panel On Hazardous Trace Substances, 1972) reviewed the transport and fate of PCB's in the environment. They note that the following processes should affect the PCB isomer ratio in the environment.

Table 40. Environmental Transport Processes of PCB's (Panel on Hazardous Toxic Substances, 1972)

Evaporation, codistillation, etc.	- reduce lower isomers near point of release increase lower isomers remote from the point of release
Photolysis	- reduce higher isomers
Metabolism & excretion	- decrease lower isomers

The ratio of higher isomers of PCB's increases downstream in some Wisconsin rivers. Kaiser and Wong (1974) have demonstrated that the less chlorinated isomers can be utilizated by bacteria. Nisbet and Sarofim concluded that the lack of lower isomers in samples from relatively contaminated areas is due to metabolism or greater mobility.

In conclusion, the importance of PCB's photolysis as an environmental degradation process has not been well demonstrated.

d. Summary

The previous discussion has pointed out our lack of understanding of photochemical and chemical alterations as environmental processes in soil and water. A large number of studies have demonstrated that numerous chemicals will be altered by light of wavelengths similar to sunlight (Plimmer, 1970; Crosby and Li, 1969). However, rarely has the relative magnitude of photochemical processes compared to other environmental degradation processes been determined. With the exception of reactions taking place in water, other non-photolytic chemical reactions catalyzed by air, heat, soils or dusts have not received a great amount of attention (Crosby, 1969a). The ubiquitous nature of water makes it an important medium for chemical reaction and considerable study has been undertaken of hydrolysis processes which take place in water. These studies have generally been limited to compounds containing esters, amides, and carbamates, although classical solvolysis reactions with heptachlor have also been noted (Rosen, 1972a). Extrapolation of these results to the environment is facilitated by the fact that the major parameter affecting the rate of hydrolysis is the pH, an easily determined parameter, both in the laboratory and environment. In contrast, in order to extrapolate photochemical results in the laboratory to reactions in the environment one needs to know the sunlight intensity and wavelength and the effect of the environmental media on light absorption and quantum yield, presence of photosensitizers, etc. With the aforementioned in mind, the following conclusions are reached.

(i) Photochemical Studies

Until the importance of photochemical processes in nature is somewhat better understood, studies of the photochemical reactivity of a compound, in terms of a routine evaluation the compounds environmental persistence and fate, should only be performed following biological and chemical degradation studies. This is recommended since we are largely unable to apply the results that are generated. In most cases, when a photodegradation product is formed with the techniques used, the best application of the results is a monitoring program to see if the compound is actually formed in the environment.

In addition, it seems reasonable that more studies should be undertaken under conditions which are likely to be important in the environment. For example, atmospheric photolysis is probably the most important photochemical process in the environment for compounds with measurable vapor pressures, and yet, so far, only one study has appeared where photolysis of a pesticide in the vapor phase has been examined (Crosby and Moilanen, 1974). In addition, air monitoring of potential photodegradation products is not available. This lack of experimentation under atmospheric conditions is probably due to the experimental difficulties involved in the vapor phase photolysis of low volatile substances. However, before the solvent and thin film techniques described in this section can be substituted for vapor phase studies, some correlation between the results needs to be established.

When a study of a chemical's photoreactivity is undertaken the experimental conditions should be varied as much as is practical in order to consider the various possible photochemical reactions. Sunlight and/or artificial light containing no wavelengths less than 290 nm should definitely be used. Light in the 300 to 400 nm range is particularly important. If the compound does not react with the low energy light sources, it might be desirable to use 254 nm light in order to determine whether the compound is at all light sensitive. The intensity of the light source should be measured and some type of rate measurement (quantum yield or at least a product study with time) should be determined. The reaction conditions should include dilute solutions in a variety of solvents (hexane, methanol, water at acidic and basic pH) as well as thin films on glass and absorption on silica gel, soil, or other solid material. Photolysis in water is particularly important since it is a major environmental medium, although isolation and identification of the products, which are usually very polar, is often very difficult. The possibility of sensitized photochemical alterations should also be investigated by running a reaction in solution (or perhaps on silica gel) with a triplet sensitizer.*

The interpretation of the results obtained must be cautiously applied. If a compound does not react under any of the conditions described above, it is pretty safe to assume that photolysis is not an important degradation process for that compound. If the compound undergoes change when exposed to >290 nm light, one may conclude that photolysis may be an important degradation process. The degrees of importance should be determined by monitoring, field, and kinetic data.

(ii) Chemical Studies

Determination of the hydrolysis rates of compounds at a variety of pH levels is extremely important, especially for compounds that are likely to reach the aquatic environment. However, the number of compounds that should undergo this type of testing can be considerably reduced by considering the chemical structure of the compound. Esters, amides, and carbamates are obvious candidates for testing. Compounds that have good leaving groups (e.g. halogens) located at positions that would stabilize a carbonium ion (allylic, benzylic, etc.) also should be tested.

The rates that are determined in distilled water may be quite different from rates in natural water. Such processes as adsorption may retard the hydrolysis rates, while on the other hand, some contaminants such as Cu^{++} may catalyze the process (Crosby, 1969a).

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* These conditions are basically those recommended by a panel of a NAS-AEC sponsored Workshop on Photoalteration of Pesticides, see Rahson and Plimmer, 1973.

With the exception of atmospheric reactions, other straight chemical processes in the environment have not received a great deal of attention. However, many of these processes (especially in soil) should be identified by the sterile blank that is run with the biological test.

5. Cost Analysis

(a) Photolysis Studies

The apparatus necessary for the techniques described previously is relatively inexpensive. A complete set of photolysis equipment can be purchased for $2-3,000. Therefore, most of the expense is due to labor and chemical analysis. Because of the degree of difficulty and the analytical procedure will vary with each compound tested, the following cost figures are only approximations. The estimations have been divided into (1) preliminary studies where a determination of photochemical loss of the compound will be provided and (2) intensive studies where major pathways are determined.

In the preliminary test, we have assumed that the compound will be studied in water, methanol, and hexane solvents, on silica gel chromatoplates, and as a thin film on glass. The analytical method will be gas chromatography, TLC, or some other technique specific for the parent molecule.

One Compound Study Only

Labor	MS/level ($15K/yr=salary + benefits)= 1 man/month	$1,250
Overhead	(125% of Professional Services)	1,560
Photolysis Equipment		3,000
TLC		500
Miscellaneous Supplies		500
	per compound	$6,710

20 Compounds/year for 5 years

Labor	MS/level 5 man/years	$ 75,000
Overhead		94,000
Photolysis Equipment		2,500
Gas Chromatograph		8,000
Miscellaneous Supplies		2,500
		$181,000
	per compound	$ 1,810

In the intensive study, we have assumed that the breakdown products were isolated by TLC or gas chromatography and were identified by mass spectrometer or GC-MS. Radiolabelled material will probably not be used unless it has been previously synthesized.

One Compound Study

Labor	MS/Level	4 man/month	$5,000
Overhead	(125% of Professional Services)		6,250
Buy GC-MS analysis (or IR, MS, NMR)			2,000
Miscellaneous supplies			500
		per compound	$11,750

10 Compounds/year for 5 years

Labor	MS/Level	5 man/years	$75,000
Overhead			94,000
GC-MS (or IR, MS, NMR) depreciated over 10 yrs.			50,000
Miscellaneous supplies			2,500
			$271,500
		per compound	$ 5,500

(b) Hydrolysis Studies

The hydrolysis breakdown products are much more predictable (e.g., ester ⟶ acid and alcohol) and, therefore, a sophisticated qualitative analysis laboratory should not be required. Again the costs are divided into (1) a preliminary study and (2) an intensive study.

For the preliminary study, we have assumed that the hydrolysis rate of the compound will be studied at acid, neutral, and basic pH and only the loss of the parent molecule will be measured.

One Compound

Labor	MS level (15K/year) = 0.25 man/months	$ 310
Overhead		390
Miscellaneous Supplies		200
	per compound	$ 900

150 Compounds/year for 5 years

Labor	5 man/year (labor/compound = .35 man/wks)	$75,000
Overhead		94,000
Gas Chromatograph		8,000
Miscellaneous Supplies		5,000
		$182,000
	per compound	$ 240

The intensive study would involve determining the hydrolysis half-life at a variety of temperatures and pH's.

One Compound

Labor	0.5 man/month	$ 620
Overhead		775
Miscellaneous Supplies		200
	per compound	$ 1595

50 Compounds/year for 5 years

Labor	5 man/year (0.25 man/month/compound)	$ 75,000
Overhead		94,000
Gas Chromatograph		8,000
Miscellaneous Supplies		5,000
		$182,000
	per compound	$ 690

VI. THE INTERCONVERSION OF ALKYLATED AND INORGANIC FORMS OF CERTAIN METALS AND METALLOIDS

A. Introduction

Elemental contaminants are introduced into the environment from various sources which include liquid and solid waste from animal and man, mining and industry, and agricultural chemicals. These elements also exist in one form or the other in the environment as natural constituents of the earth's crust. Elements which are of major concern today include: mercury, arsenic, lead, copper, cadmium, chromium, nickel and vanadium. These exist in the environment in many different forms as air pollutants, contaminants of water and soil, and as residues in food.

Pollution of our environment by mercury and its conversion to poisonous methyl mercury by microorganisms has caused concern over the effect of such trace metals. The toxic metals in the environment may present a more insidious problem than pollution by organic chemicals because metals, unlike organic chemicals, cannot be degraded to innocuous products such as carbon dioxide and water. The degradation of organometallic compounds by microorganisms generally leads to the liberation of toxic elements in addition to other end products (Nelson *et al*., 1973; Von Endt *et al*., 1968). Metals can undergo changes in valance state, be converted into organometallic form or be mobilized from one environment to another. The interconversions are generally reversible and result in steady state concentrations of various forms in the environment.

Heavy metals have been known to be toxic to humans, animals and plants. The form in which the metal occurs (e.g., pure metal, inorganic compound, or organometallic compound) strongly influences its toxicity. A small disturbance in the dynamic biological and chemical cycling of these toxic elements can have considerable impact on their concentrations in the environment.

In view of the toxic nature of certain elements, it becomes important to know the fate of these chemicals in the environment. In the case of metals and metalloids, the mechanisms involved in their transport and transformation from one form to another are extremely important from the point of view of environmental pollution. In reviewing the test methods which have been used to study the transformation and fate of heavy metals and their compounds in the environment, the organization used in previous sections has been slightly modified. Realizing the differences in the type and nature of the reactions involved in heavy metal transformations, it is necessary to review first the possible chemical and biochemical transformation of selected elements in the environment.

B. Chemical and Biochemical Transformation of Metals and Metalloids

Microorganisms, especially bacteria and fungi, play an important role in catalyzing the modification, activation, or detoxification of compounds of the toxic metals and metalloids. These interconversions could yield a product which may be more or less toxic to higher organisms. The pathways of elemental and organometallic forms of heavy metals are the following:

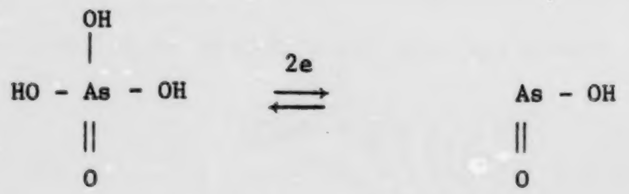
1. Valance Changes:

Trace metals can exist in the environment in several valance forms and each form may differ in its toxicity. By means of oxidative and reductive reactions catalyzed by biological or chemical means, the relative abundance of each species is controlled. For example, mercury can exist in the following three forms (Wood, 1974):

$$\mathrm{Hg_2^{2+} \rightleftarrows Hg^{2+} + Hg^{\circ}}$$

The relative amounts of each form will depend on the solubility or the amount of dissociation of the mercuric compound formed and the extent to which metallic mercury enters or leaves the system. Vaporization of elemental mercury will shift the reaction to the right, whereas an increase in the concentration of $\mathrm{Hg^{\circ}}$ either due to return from the atmosphere or due to addition from external sources will shift the equilibrium to the left. These transformations are also catalyzed by microorganisms (Wood, 1974).

Microorganisms are able to reduce the pentavalent form (arsenate) of arsenic to the trivalent form (arsenite) (McBride and Wolf, 1971). On the other hand, a number of microorganisms are able to oxidize arsenite to less toxic arsenate (Mendal and Mayersak, 1962; Turner, 1954; Turner and Legge, 1954).



$$\mathrm{HO-\underset{\underset{\displaystyle O}{\|}}{\overset{\overset{\displaystyle OH}{|}}{As}}-OH \quad \overset{2e}{\rightleftarrows} \quad \underset{\underset{\displaystyle O}{\|}}{As}-OH}$$

2. Methylation:

Biological methylation plays an important role in the transport of toxic metals and metalloids. Researchers have reported methylation of mercury, arsenic, selenium and tellurium. Conversion of inorganic forms of metal or metalloids to methylated forms is sometimes employed by microorganisms as a detoxification mechanism.

Mercury: Jensen and Jernelov (1969), and Fagerstrom and Jernelov (1971), have reported formation of both monomethyl and dimethyl mercury in lake and river sediments. Bisogni and Lawrence (1973) have pointed out that biological wastewater treatment systems and anaerobic digesters may provide excellent environment for methylation of mercury. Wood and coworkers (1968) have investigated the biochemical pathways for synthesis of methylmercury compounds. These investigators have also reported that transfer of the methyl group from Co^{3+} of methylcobalamine to Hg^{2+} could be catalyzed nonenzymatically under mild reducing conditions.

Dimethylmercury resulting from microbiological transformation can be transported to the atmospheric environment due to its volatility, where it may be subjected to photolysis. According to Gomer and Noyes (1949) the photolysis of dimethylmercury might proceed as follows:

$$Hg\,(CH_3)_2 \xrightarrow{hv} CH_3^{\cdot} + \cdot HgCH_3$$

The monomethyl mercuric radical can further undergo decomposition to give rise to metallic mercury and methyl radical. The methyl radicals probably abstract hydrogen or recombine to form methane or ethane, respectively (Wood, 1974).

Spangler and coworkers (1973a, 1973b) have reported evidence for the microbial degradation of methylmercury in lake sediments. It was suggested that organisms responsible for degradation of methylmercury may serve a useful purpose in maintaining the environmental methylmercury concentrations at a minimum. The bacterial species isolated in pure culture caused conversion of methylmercury into volatile elemental mercury (Hg^0) and methane.

The biochemical, chemical and photochemical transformations of mercury compounds set up a dynamic system in the environment. The biological cycle for mercury suggested by Wood (1974) is shown in Figure 46.

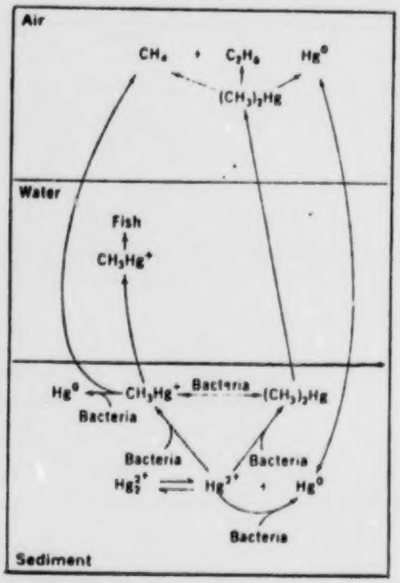

Air
CH₄ + C₂H₆ Hg⁰
(CH₃)₂Hg
Water
Fish
CH₃Hg⁺
Hg⁰
CH₃Hg⁺
Bacteria
(CH₃)₂Hg
Bacteria
Bacteria
Bacteria
Hg₂²⁺
Hg²⁺ + Hg⁰
Bacteria
Sediment

Figure 46. The Biological Cycle for Mercury
(Wood, 1974)
Courtesy of J.M. Wood, Science, 183(4129), 1049-52
Copyright 1974 by the Amer. Assoc. for the Advancement of Science

Arsenic: Arsenic, similar to mercury, has also been found to undergo methylation. Microorganisms, particularly bacteria and fungi, have been reported to play active roles in these conversions (McBride and Wolf, 1971; Challenger, 1945; Cox and Alexander, 197). The biochemical pathway leading to the formation of methylated forms of arsenic have been investigated by McBride and Wolf (1971). In the scheme proposed, arsenate (+5) is first reduced to arsenite (+3), which is methylated to form methylarsenic acid. The latter component is reductively methylated, forming dimethyl and trimethyl arsine. Because of their volatility, alkylarsines may appear in the atmospheric environment where they may be rapidly oxidized. Kearney and Woolson (1973) have reported alteration of organoarsenic compounds via two pathways: (1) an oxidative pathway leading to C-As bond cleavage and (2) a reductive pathway leading to alkylarsine production. The biological cycle of arsenic is shown in Figure 47.

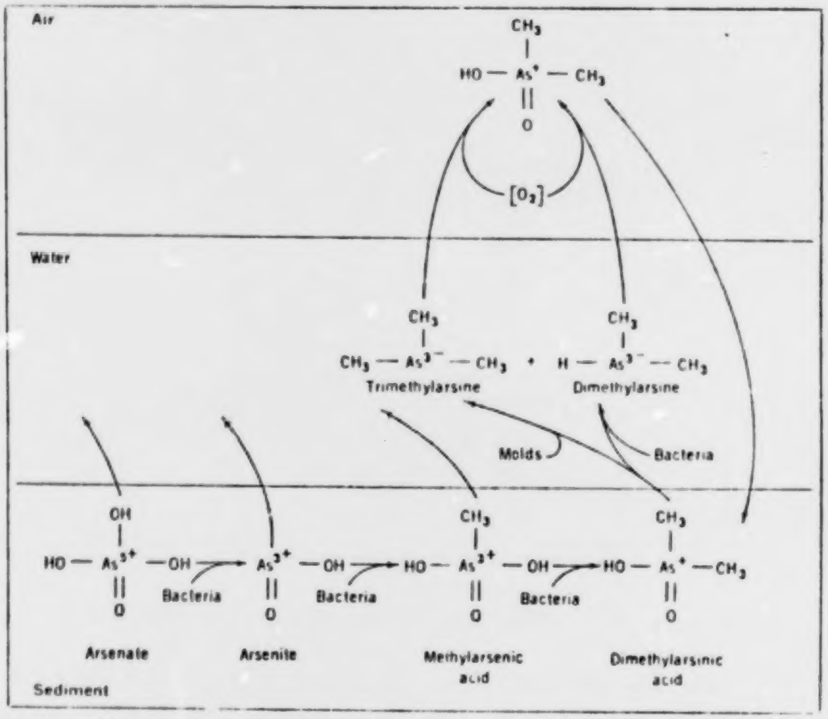

Air
CH₃
HO — As⁺ — CH₃
O
[O₂]
Water
CH₃
CH₃ — As³⁻ — CH₃
Trimethylarsine
CH₃
H — As³⁻ — CH₃
Dimethylarsine
Molds
Bacteria
OH
HO — As⁵⁺ — OH
O
Bacteria
As³⁺ — OH
O
Bacteria
CH₃
HO — As³⁺ — OH
O
Bacteria
CH₃
HO — As⁺ — CH₃
O
Arsenate
Arsenite
Methylarsenic acid
Dimethylarsinic acid
Sediment

Figure 47. The biological cycle for arsenic (Wood, 1974)

Courtesy of J.M. Wood, Science, 183(4129) 1049-52
Copyright 1974 by the Amer. Assoc. for the Advancement of Science

Selenium and Tellurium: Selenium and tellurium have been known to be acted on by microorganisms to produce methylated compounds. Fleming and Alexander (1972) isolated a strain of Penicillium from raw sewage which produced dimethylselenide from inorganic selenium compounds. The same organism also catalyzed the conversion of several tellurium compounds to dimethyltelluride. The methylation of selenium was later also shown to occur in soil (Alexander, 1973c).

3. Chelation

Natural and man-made chelates, both organic and inorganic, occur everywhere in the environment at low concentrations. One example of this is trisodium nitriloacetic acid, which is contemplated as a possible detergent additive. This could lead to an increase in the concentration of the chelant in water and may play an important role in making metals more soluble and, therefore, accessible, by forming chelates. The anion forms of NTA can react with appropriate metal ions to produce the metal chelate (Thom, 1971):

$$NTA^{3-} + M^{2+} \rightleftharpoons NTA - M^{-}$$

Swisher et al. (1973) have indicated that NTA metal chelates are biodegradable and, therefore will not be expected to accumulate in the environment. However, the actual contribution of metal chelation to the biological cycle of toxic elements in the environment is largely unknown.

C. Test Methods for Studying Transformation

1. Biological Transformation in Aquatic Environment

a. Methylation of metals

(i) Mixed Culture Studies: Biological methylation of mercury compounds by aquatic organisms was first studied by Jensen and Jernelov (1969). They treated 1 gm samples of bottom sediments from fresh water aquaria with $HgCl_2$ (100 ppm). Untreated samples and suspensions sterilized by autoclaving served as controls. Samples were incubated for 5 to 10 days and then analyzed for CH_3Hg^+. The formation of dimethylmercury from mercury or from monomethylmercury was studied using homogenates of dead (rotten) fish (Xiphophorus maculatus) as the biological material. The conversion was studied under anaerobic conditions, which were obtained by flushing the flasks with nitrogen. Bishop and Kirsch (1972) studied the biological generation of methylmercury under anaerobic conditions using a tertiary sewage lagoon and a methanogenic enrichment culture as the microbial source.

In order to study the mercury transformations in the aquatic environment, Bisogni and Lawrence (1973) constructed microbial reactors which could be operated under anaerobic (low redox potential) and aerobic (high redox potential) conditions. In both phases the units are operated on a semicontinuous basis. Inorganic mercury in the form of mercuric chloride (concentrations varying from 0.1 to 100 mg/l of mercuric ion) is introduced with the feed solution. The feed solutions for the microbial reactors consisted of carbonaceous-nitrogenous media (nutrient broth-glucose mixture), nutrient salts, buffer salts and tap water. Since the nutritional requirements for anaerobic and aerobic microbes are somewhat different, two different inorganic salt solutions were used. The nutrient salt solution of McCarty and Speece (1963), supplemented with $CoCl_2$, was used for anaerobic cultures and the nutrient salt solution of Bisogni and Lawrence (1973) was used for aerobic cultures. Another difference between the two systems was that for anaerobic units a bicarbonate buffer system was used, whereas the aerobic units used a phosphate buffer system. In order to verify that methylation reactions were mediated by microorganisms, sterile reactors were run parallel to microbial reactors.

The anaerobic microbial reactor used by Bisogni and Lawrence (shown in Figure 48) was constructed from a 1 or 2 liter Erlenmeyer flask, and was equipped with a dimethylmercury trap, a mercury scrubber system, and a gas measuring cylinder. The system was inoculated with the seed obtained from an anaerobic digester at a municipal sewage treatment plant.

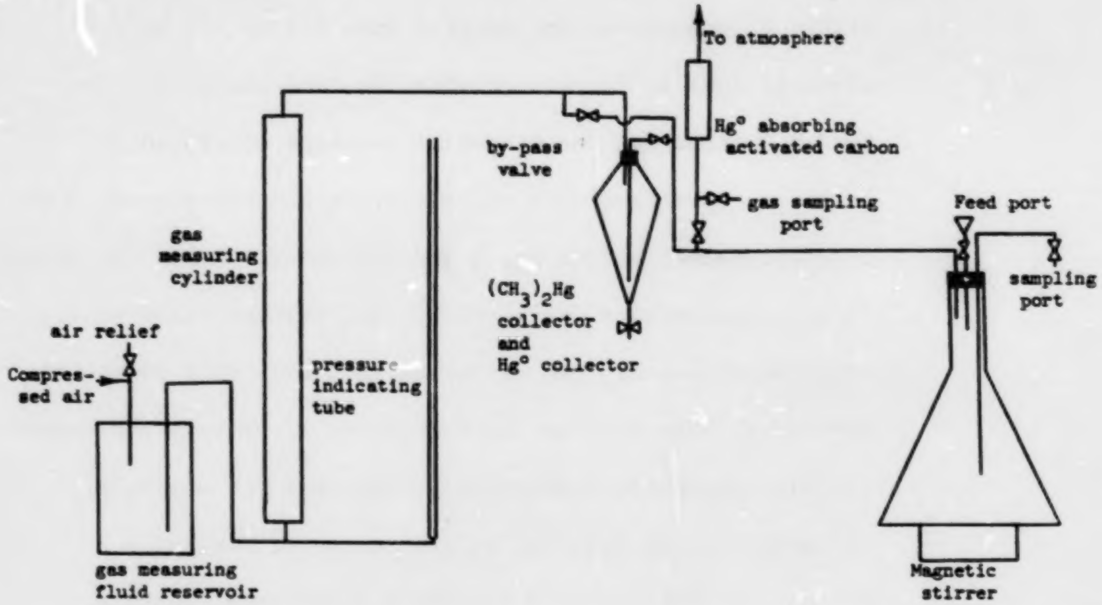

To atmosphere
Hg° absorbing activated carbon
by-pass valve
gas sampling port
Feed port
sampling port
gas measuring cylinder
(CH3)2Hg collector and Hg° collector
air relief
Compressed air
pressure indicating tube
gas measuring fluid reservoir
Magnetic stirrer

Figure 48. Anaerobic Microbial Reactor System (Bisogni and Lawrence, 1973)

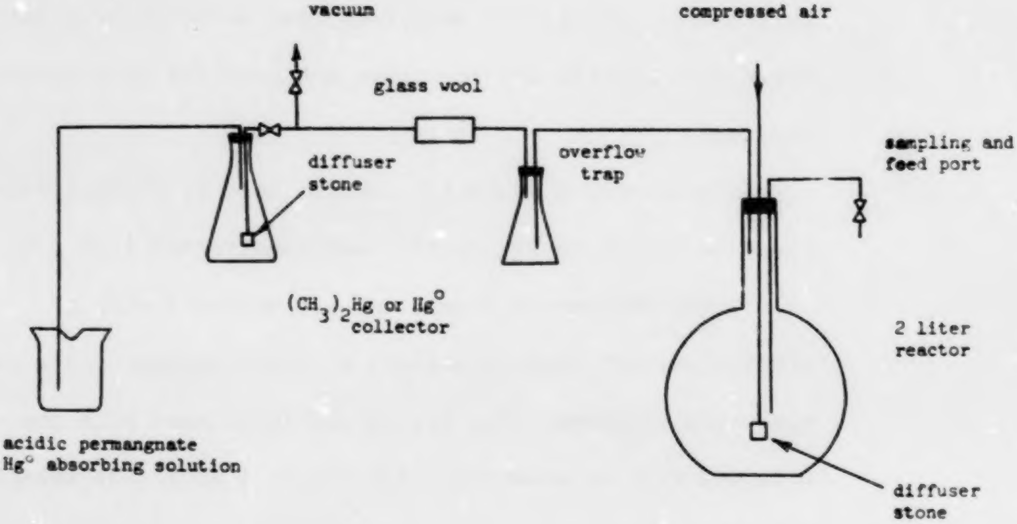

vacuum
compressed air
glass wool
diffuser stone
overflow trap
sampling and feed port
(CH3)2Hg or Hg° collector
2 liter reactor
acidic permangnate Hg° absorbing solution
diffuser stone

Figure 49. Aerobic Microbial Reactor System (Bisogni and Lawrence, 1973)

Their aerobic microbial reactor was constructed from a 2 liter Florence flask (Figure 49). Compressed air as a source of oxygen was supplied through a gas diffuser stone. A dimethylmercury trap and a metallic mercury vapor trap were attached to the flask as shown. The seed for the aerobic units was obtained from the aeration chamber of a municipal sewage treatment plant and maintained on a fill and draw basis for a period of three months in the laboratory prior to the use in the aerobic reactor.

(ii) Pure Culture Studies - Intact Cells and Cell-Free Extracts: Tonomura _et_ _al_. (1972) have studied the formation of methylmercury by an anaerobic bacterium (_Clostridium_ _cochlearium_) isolated from the soil. The organism was incubated anaerobially (gas phase, nitrogen) in a medium containing organic nutrients, cysteine, mercuric chloride and vitamin B_{12}, and the methylmercury formed was assayed. McBride and Wolfe (1971) studied the biological formation of alkylarsines utilizing pure cultures of a methanogenic bacterium (_Methanobacterium_ strain M.O.H.). This bacterium was chosen since the extracts of this bacterium were earlier shown to catalyze the formation of methylmercury from inorganic mercury (Wood _et_ _al_., 1968). In order to assay for the formation of alkylarsine, whole cells of the bacterium were incubated with sodium arsenate in a gas atmosphere of H_2-CO_2 (80:20). The temperature was kept at 40°C.

Fleming and Alexander (1972) studied the formation of dimethylselenide and dimethyltelluride by a strain of Penicillium isolated from raw sewage. The fungus was isolated by plating on the medium containing inorganic salts and an organic carbon source as well as Na_2SeO_3 (1000 μg/ml). The ability of the isolated fungus to form the volatile products (dimethylselenium and dimethyltellurium) was investigated by inoculating the medium (defined medium or autoclaved municipal sewage) containing sodium selenite with the washed spore suspension of the fungus. The vessels were capped with a foam plug during the first 72 hours and later with serum stoppers (since the synthesis of dimethylselenide was found to start after 72 hours). Gas samples were withdrawn at various intervals and the volatile products were assayed. Vonk and Sijpesteijn (1973) studied the methylation of mercury during aerobic growth of several bacterial and fungal species which are commonly found in water and soil. These researchers used a sublethal concentration of mercuric ion in their experiments (range between 5-20 mg/ℓ).

Wood et al. (1968) have studied the synthesis of methylmercury compounds using the extracts of methanogenic bacterium (Methanobacterium strain M.O.H.). The organism was grown on hydrogen and carbon dioxide (80:20). The reaction mixture for the assay of the formation of methylmercury and dimethylmercury contained crude extracts, adenosine triphosphate (as energy source), Hg^{2+}, methylcobalamine and potassium phosphate buffer (pH 7.0). The gas phase was hydrogen which served as the source of electrons. Assay for methylated forms of mercury was performed after an incubation period of 50 minutes.

McBride and Wolfe (1971) used the extracts of Methanobacterium strain M.O.H. to study the formation of dimethylarsine from sodium arsenate. The reaction conditions were the same as those used by Wood et al. (1968). A reaction flask which was heated in a boiling water bath prior to incubation was run parallel to the experimental flask to determine the extent of chemical methylation of arsenic.

(iii) Field Studies: Jacobs and Keeney (1974) studied methylmercury formation and Hg loss from mercury-treated river sediments during in situ equilibration. Bulk sediments were collected from river sites, treated with mercuric chloride or phenylmercuric acetate (approx. 1-100 ppm Hg, oven-dry basis) and returned to the river with untreated controls. After equilibration with the river environment for various intervals (2-12 weeks) samples were removed for analysis.

Kania et al. (1973) have investigated the fate of mercury in artificial stream channels 300 ft. long, 2 feet wide and 1 ft. deep. The flow of water was set with manual control valves to provide 25 gallons per minute into each channel. The average retention time in the stream was 2 hours. Feeding was allowed to occur naturally in the channels except that mosquito fish were introduced a month before mercury was added. The levels of mercury were low (1 and 0.01 ppb). Fish and the sediment samples were analyzed for the total mercury at various intervals.

b. Degradation of Organometallic Compounds

Spangler *et al*. (1973a) have studied the bacterial degradation of methylmercury in lake sediment. They incubated sediment water mixture to which was added $HgCl_2$ under aerobic conditions to permit the formation of methylmercury; an aliquot from this flask was used to inoculate the medium containing methylmercury in tryptic soy broth and the mixture was incubated for 170 hours. Uninoculated controls containing methylmercury were run simultaneously. These researchers later obtained pure cultures of aerobic and anaerobic microorganisms capable of degrading methylmercury, by direct isolation or isolation from enrichment cultures (Spangler *et al*., 1973b).

Swisher *et al*. (1973) studied the biodegradation of NTA metal chelates in river water collected from the Meramec River. NTA was added at a concentration of 5 mg/ℓ. Selected metals were added separately as chlorides at three levels (equivalent to 5, 1.5 and 0.5 mg/ℓ NTA). Another sample was set up with NTA and no added metal.

A few reports are available in the literature in which researchers have utilized the mercury-resistant bacteria in biodegradation studies of organic mercurial compounds such as phenylmercuric acetate (Nelson *et al*., 1973; Furukawa *et al*., 1969). Furukawa *et al*. (1969) studied the decomposition of phenylmercuric acetate by a strain of bacteria named K62, belonging to the genus *Pseudomonas*, which has been shown to be resistant to organic and inorganic mercury compounds (Tonomura et al., 1968). Cells grown on a medium containing organomercurial compounds were incubated with the test mercury compound (10-100 ppm) in phosphate buffer and decomposed products were analyzed. Nelson et al. (1973) isolated strains of mercury-resistant bacteria, which could degrade phenylmercuric acetate, from estuarine water and sediment samples by plating on a medium containing phenylmercuric acetate and mercuric chloride.

2. Biological Transformation in the Soil Environment

The degradation work in the soil environment has mostly been restricted to organo-arsenical pesticides. Researchers have used many different types of soil in these studies. The decomposition of methanearsonate (MSMA) was studied by Dickens and Hiltbold (1967) in 4 different soils: Norfolk loamy sand, Augusta silt loam, Decatur clay loam and Vaiden clay. Methanearsonate was added to 20 gm samples of soil to give a concentration of 110 ppm. Herbicide decomposition was also investigated in the presence of added decomposable organic matter. The soils were moistened to field capacity and degradation was studied up to 80 days. Von Endt et al. (1968) in their studies on the degradation of methanearsonates used the following soils: Sharkey clay, Hagerstown silty clay loam, Cecil sandy loam and Dundee silty clay loam. The concentration of MSMA ranged between 10 and 100 ppm, and incubation with soil was continued for up to two months. Steam sterilized soils were also incubated with methanearsonate to determine the chemical breakdown.

For studying persistence of cacodylic acid, Woolson and Kearney (1973) used three soils: Lakeland loamy sand, Hagerstown silty clay loam and Christiana clay loam. The soils in these studies were brought to 75% field capacity and incubated for 32 weeks. The incubation was carried out under aerobic as well as anaerobic conditions.

Von Endt et al. (1968), using soil enrichment technique (see section IV.A.1.b., p. 231) isolated a fungus, several actinomycetes and several bacteria capable of metabolizing methanearsonate. The concentration of methanearsonate (monosodium salt) during enrichment was 100 ppm. Experiments involving degradation of arsenicals were conducted in Roux bottles. Solidified universal salt solution (Kearney et al., 1964) containing 3% agar and 100 ppm methanearsonate was inoculated with the isolates of soil microorganisms and degradation was studied.

3. Model Ecosystem and Aquarium Studies

Aquatic ecosystems which simulate the aquatic environment have been used in studies concerning fate and transformation of heavy metals and metalloids. Bahr and Ball (1969) in their studies on arsenic metabolism used separate aquariums for groups of similar organisms as well as a complete ecosystem. Aquariums containing sand, pond mud or gravel and water with plants, fish or invertebrates each in separate aquariums were used to study the fate of arsenicals in the absence of interference by other factors. A complete ecosystem containing 12 plants each (Potamogeton, Elodea and Isoetes sp.), 6 green sunfish, 6 fat head minnows, 6 bull-heads and 6 of each invertebrates (crayfish, snails, and dragonfly naiads) was also used by these researchers to study the metabolism of arsenic. The ecosystem, described above, appears to be more oriented towards the higher organisms since nothing is mentioned about the extent of any microbial transformation in these studies.

Fang (1973) has studied the biotransformations of phenylmercuric acetate in aquatic organisms. Studies were performed in an aquarium containing guppies, snails (genus Helisoma), elodea and coontail. Again there is no mention about any microbial transformation in their studies also. Isensee et al. (1973) have used a modified version of Metcalf's model ecosystem (see section III.A.5., p. 118) to study the distribution and fate of alkylarsenicals. The terrestrial phase of the ecosystem of Metcalf et al. (1971) was omitted but 10 grams of soil was added to treated and control (untreated) tanks. ^{14}C-cadocylic acid was added directly to the tanks while ^{14}C-dimethyl arsine was first absorbed to the soil and then added to the aquarium. In view of the fact that cacodylic acid is used predominantly in controlling pests of cotton, Schuth et al. (1974) reexamined the fate of cacodylic acid in micro-ecosystem which contained bottom feeding organisms (catfish and cray fish - indigenous to cotton producing areas) and duckweed (Lemna minor L), daphids and snails. The pesticide was mixed with soil prior to the addition to the tank.

The fate of cadmium in an experimental ecosystem was investigated by Gaskins et al. (1971). These researchers used an ecosystem (also called microcosm) which contained soil, water, plants, and fresh water fish and snails (names of species not mentioned) from a plot of land near the laboratory. The radioactive cadmium was added to the terrestrial part of the ecosystem as a component of a single, simulated rainfall.

4. Test Methods for Photochemical Studies

For studying photochemical transformations of organomercurials, Baughman et al. (1973) and Zepp et al. (1973) irradiated samples by:

a. Broad-band (>290 nm) and monochromatic (313nm) light from a mercury lamp. The light was filtered through a pyrex sleeve for the broad band studies and through a pyrex sleeve and a solution of potassium chromate in aqueous potassium carbonate to isolate the 313nm line.

b. Sunlight. Aqueous solutions (10^{-4}M) of methylmercury compounds were degassed in quartz tubes which were sealed and irradiated by September sunlight for approximately 17 hours.

In order to study sunlight photolysis of phenylmercury compounds (concn. range 4 x 10^{-5} to 10^{-6}M in water) samples were exposed to approximately 20 hours of sunlight. Acetone-sensitized photodecomposition of phenylmercury compounds was investigated at pH 2.3 and pH 10.2. The samples were irradiated by pyrex-filtered light (>290nm).

5. Test Methods for Studying Chemical Transformation

Baughman _et al_. (1973) have studied the acidolysis of dimethylmercury utilizing two procedures: in one method, dimethylmercury was added to an aqueous solution of tetrahydrofuran (the GLC internal standard); a standardized acid solution was added and the mixture was transferred to a kinetic bomb. The bomb was immersed in thermostated oil bath and periodically aliquots were removed and analyzed by GLC. In another method, the acid solution was added to dimethylmercury solution under nitrogen in a glove box. The solution was transferred to a conductance cell immersed in a thermostated water bath and conductance of the solution was recorded at appropriate intervals.

In order to investigate the chemical methylation of mercury, Wood _et al_. (1968) incubated methylcobalamine and propylcobalamine with two individual batches of Hg^{2+} under mild reducing conditions and the resulting products were identified.

D. Analytical Procedures

Transformation of metals and metalloids, like other environmental contaminants, has been studied either by assaying for the disappearance of the parent compound or by following the formation of the metabolites and products. The volatility of the several intermediate metal compounds and their tendency to adsorb on particles and surface may sometimes present a problem in accuracy. The analytical procedure employed for studying metal transformation usually involves following three steps:

1. Isolation Steps

The choice of extractant is, perhaps, one of the critical steps in any analysis. Volatile metabolites of heavy metals are usually first trapped in a suitable mixture and then recovered by extraction with organic solvents. $HgBr_2$-KBr solutions are generally employed as the trapping solution. This is particularly suited for trapping mercury vapors or organomercurials (dimethylmercury) (Spangler et al., 1973a; Spangler et al., 1973b). A $Hg(NO_3)_2$-HNO_3 trapping solution is equally effective in trapping, but is not widely used due to low extraction efficiency for organomercurials (Spangler et al., 1973b). Volatile alkylarsines have commonly been trapped by oxidation to nonvolatile acids by treatment with nitric acid which is kept in ethanol-ice baths (Raiziss and Garron, 1923; McBride and Wolf, 1971). Other researchers have used silver diethyldithiocarbamate-pyridine solution for trapping alkylarsines (Powers et al., 1959; Sachs, et al., 1971). A second method based on the property of alkylarsines to react with the red-rubber stopper used to seal the reaction vessel has also been used (McBride and Wolf, 1971). For extraction of organic mercury, the benzene-cysteine extraction technique developed by Westoo (1967) is frequently used (Fang, 1973; Bisogni and Lawrence, 1973; Vonk and Sijpesteijn, 1973). A few researchers have preferred to use toluene instead of benzene for extraction (Spangler et al., 1973a, 1973b). Inorganic mercury is not extracted into the organic solvent phase and, therefore, provides no interference. The recovery in benzene-cysteine extraction technique is 98 ± 3 percent. The technique can also be used to measure phenylmercury (Gage, 1961). For measurement of dimethylmercury, the gas samples containing dimethylmercury are passed through a solution of mercuric chloride in 2N HCl prior to subjecting to benzene-cysteine extraction. For extraction of mercury from fish, sulfuric acid:nitric acid mixtures have been used. Sediment samples are autoclaved to release mercury (Kania _et_ _al_., 1973).

2. Analysis

The following analytical procedures have been used for studying trace metal transformations:

a. _Gas chromatography_:

The method is used widely for determining trace amounts of volatile metabolites of heavy metals (Fleming and Alexander, 1972; Jensen and Jernelöv, 1969; Spangler _et_ _al_., 1973 a & b; Vonk and Sijpesteijn, 1973). Methylated forms are analyzed by gas chromatographic detection of CH_3MX or CH_3MCH_3 (where M = metal, X = halogen) using an electron capture detector. Gas chromatography has also been used in trace metal analysis in which case a volatile fluoroacetylacetonate derivative of the metal is prepared (Ross et al., 1965; Bayer et al., 1971; Lisk, 1974). Dimethyl intermediates may be first converted to monomethyl derivates prior to injection in the gas chromatograph in order to increase the sensitivity of the electron capture detector. The technique is very sensitive and can be used to separate and detect metabolites as well as the parent compound.

b. Use of labelled compounds:

One of the simpler methods to study the transformation of heavy metals is by the use of radioactive elements or organometallic compounds labelled in the element or in the carbon. Researchers have used ^{14}C-labelled compounds for studying the fate of arsenicals in soil and in model ecosystems (Von Endt et al., 1968; Dickens and Hiltbold, 1967; Woolson and Kearney, 1973; Isensee et al., 1973). ^{74}As-labelled compounds have been utilized by McBride and Wolf (1971), Bahr and Ball (1969). In studies concerning the degradation of organomercurials ^{203}Hg or ^{14}C-labelled compounds have been used (Fang, 1973; Furukawa et al., 1969; Nelson et al., 1973). Radioactive metabolites formed are separated by thin-layer chromatography and may be identified by cochromatography. Preparative chromatography can be used to obtain small quantities of material for further identification of unknown metabolites. The radiolabelled metabolites can be more easily detected and identified.

c. Atomic absorption:

In conventional flame atomic absorption spectrometry, the specific element is burned in the flame and the energy absorbed at a specific wavelength is measured. The conventional flame emission or absorption spectrometry is not widely used primarily because of the availability of newer techniques which afford superior sensitivity. A recently-developed technique referred to as flameless or cold vapor technique, involves chemical reduction of test metal to the elemental form, its volatilization into a long-path-length absorption tube and measurement of the absorption at appropriate wavelengths. The method is both sensitive (detection limit 0.2 ppb for mercury, 0.5-1.0 ppm for arsenic, Wallace et al., 1971; Braman and Foreback, 1973) and rapid, and has been used in heavy metal analysis by Iskandar et al., 1972; Jacobs and Keeney, 1974; Hatch and Ott, 1968; Bisogni and Lawrence, 1973. Chemical agents such as benzene, toluene, xylene, chloride interfere with the method and can produce positive absorbance peaks. Limits of detection for various elements in this method are given in Table 41.

Table 41. Absolute Limits of Detection (in g) for Atomic Spectrometric Methods (Karasek and Laub, 1974)

Element	Atomic absorption	Atomic fluorescence	Atomic emission
Ag	10^{-10}	10^{-11}	10^{-8}
As	10^{-8}	10^{-8}	10^{-5}
Cd	10^{-10}	10^{-13}	10^{-6}
Cu	10^{-9}	10^{-10}	10^{-9}
Hg	10^{-7}	10^{-10}	10^{-5}
Zn	10^{-9}	10^{-11}	10^{-5}
Pb	10^{-9}	10^{-9}	10^{-7}

d. Spectrophotometric procedure:

The diphenylthiocarbazone (dithizone) method is probably the most widely used colorimetric method for detection of trace levels of mercury.

In this method, dithizone is reacted with Hg_2^{2+} or Hg^{2+} in acid solution to form a colored complex which is extracted with chloroform or carbontetrachloride (Sandell, 1959; Snell and Snell, 1949). The absorption of the complex is measured at 490nm or alternatively the decrease in dithizone absorbance at 610nm is measured. The procedure can be used to determine 0.5 to 50 ppm of mercury. Presence of copper, silver, gold, etc. interferes with the determination. Preliminary treatment to insure decomposition of organic material is required in order to determine organic-bound mercury by this method.

The silver-diethyldithiocarbamate (AgDDC) colorimetric method has been used for determination of arsenic (Powers _et al_., 1959; Dickens and Hiltbold, 1967). In this method arsenate is first reduced to arsenite, which is then reacted with hydrogen to form gaseous arsine. Arsine is trapped in silver diethyldithiocarbamate-pyridine solution and the stable red complex formed is measured at 540nm. The lower limit of detection for this method is 2 ppb (Braman and Foreback, 1973). The method can be used for determination of arsenic in organic arsenicals. However, the carbon-arsenic bond should be first ruptured by digestion with nitric acid-sulfuric acid oxidation procedure (Sachs _et al_., 1971).

e. _Neutron activation_:

This procedure can be used for determination of several elements with great sensitivity. The technique involves exposing a sample to a source of neutrons to produce a radioactive nuclide of the element (Lyons, 1964). For mercury, for example, irradiation results in 2 radioactive nuclides, ^{197}Hg and ^{203}Hg. Although the method is extremely sensitive, its use has so far been restricted to monitoring only for trace levels of metals in environmental samples. The major disadvantage of this technique is that it requires special irradiation facilities and data handling.

f. X-ray fluorescence:

In this method low-energy photons are used to excite the characteristic x-ray energy of the element (Wallace et al., 1971). The x-rays emitted are then sorted and measured using a solid-state Ge(Li) or Si detector coupled to a multichannel analyzer.

g. Polarographic Methods:

This technique is more suitable as a supplementary and reference method, since its sensitivity is not high enough for use in analysis of environmental samples (Wallace et al., 1971).

Limits of detection of various methods:

The well-known instrumental methods which have been used in studying transformation of metals are summarized in Table 42.

3. Identification

Thin-layer chromatography is one of the commonly used techniques for separation and identification of mercury compounds. Most frequently researchers have used silica gel and alumina thin-layer plates (Westoo, 1969; Tatton and Wagstaffe, 1969). Johnson and Vickers (1970) have used thin-layer plates to separate various organic mercurials and inorganic mercury. In several studies organomercurials have been reacted with dithizone to form dithizonate complexes prior to separation by thin-layer chromatography. Von Endt et al. (1968) have used thin-layer plates coated with silica gel G (calcium sulfate binder) to separate various inorganic and organic arsenicals.

Table 42. Instrumental Limits of Detection in Trace Metal Analysis
(Karasek & Laub, 1974)

Method	Uses	Minimum Detectable Quantity, g	Price Range, $
1. Gas chromatography (GC)			500 to 10,000
A. electron capture	electrophilic organics e.g. CH_3HgCl	10^{-13}	
B. helium ionization	all volatile compounds	10^{-13}	
2. Mass spectrometry (MS)			20,000 to 250,000
A. spark source	all elements, most compounds	10^{-13}	
B. electron impact--direct probe	all elements, most compounds	10^{-12}	
C. GC/MS/computerized		10^{-11}	
D. ion-probe	all elements, some inorganics and organics	10^{-15}	
3. Neutron activation analysis (NAA)	all elements	10^{-12}	>250,000
4. Atomic absorption spectroscopy (AAS)			500 to 20,000
A. flame	metallic elements	10^{-9}	
B. flameless	metallic elements	10^{-12}	
5. Electron spectroscopy (ESCA)	most elements, some compounds	10^{-7}	60,000 to 250,000
6. X-ray fluorescence	elements with atomic number >12	10^{-7}	50,000
7. Ion-scattering spectrometry (ISS)	all elements, some inorganics and organics	10^{-15}	40,000 to 65,000
8. Polarography	most metallic elements and compounds, some inorganics	10^{-6}	500 to 1,500

The compounds were identified by cochromatography. A few researchers have separated the arsenicals by electrophoresis on chromagram cellulose plates (McBride and Wolf, 1971).

Combined gas chromatography-mass spectrometry has been extensively used for detection of metabolites (Fleming and Alexander, 1972; Jensen and Jernelöv, 1969). The technique has an almost universal ability to separate components and provide a mass spectrum for identification as well as detection. Overall fragmentation pattern of authentic methylated form of the trace metal can be compared with that of unknown compounds for identification. In certain cases, however, absorption of the chemical on the large surface area in GC/MS systems and thermal decomposition may greatly reduce the applicability of this technique.

E. Evaluation of Techniques

1. Factors Affecting Transformation of Elemental Contaminants

a. Factors Affecting Methylation of Metals

Factors which influence the methylation of metals are generally the same as those which affect other microbiological transformations. These include the concentration of the metal, number and type of microorganisms present and their growth rates, adsorption and chelation of the metal, presence of other chemicals including supplemental nutrients, physical parameters such as pH, temperature, and redox potential of the test medium.

(i) Concentration of the metal: Jensen and Jernelöv (1969) studied the formation of methylmercury under aerobic conditions as a function of inorganic mercury ($HgCl_2$) concentrations in the lake sediment. Their findings indicated (Figure 50) that

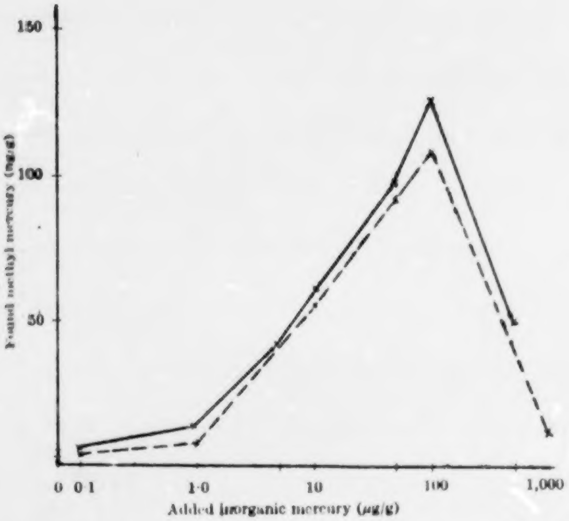


Figure 50. Concentration of Methylmercury in Bottom Sediment After Addition of Inorganic Mercury Followed by Incubation for Seven Days (Jensen and Jernelov, 1969). Two parallel experiment series were run, to the first series 0, 0.1, 1, 10, 100 and 1000 ppm $HgCl_2$ and to the other 1, 5, 10, 50, 100 and 500 ppm $HgCl_2$ were added.
Courtesy of Nature.

methylmercury production increased with increasing inorganic mercury dosage up to 100 µg/g of sediment. A further increase in inorganic mercury caused a sharp decrease in the formation of methylmercury, probably due to the inhibition of the methylating microorganisms. An increase in methylmercury formation with increasing inorganic mercury concentrations has also been observed under anaerobic conditions (Bishop and Kirsch, 1972; Bisogni and Lawrence, 1973). Fleming and

Alexander (1972) studied the formation of dimethylselenide from inorganic selenium. These investigators found that in the medium containing 10 μg selenite/ml, as much as 13-24% of the added selenite was converted to dimethylselenide, whereas at 1000 μg selenite/ml, less than 2% of the selenium was recovered in the dimethylated form.

(ii) Microbial activity: Methylation of metals has been demonstrated to be catalyzed by anaerobic as well as aerobic bacteria (Tonomura _et al_., 1972; Vonk and Sijpesteijn, 1973; McBride and Wolfe, 1971), and fungi (Fleming and Alexander, 1972; Cox and Alexander, 1973; Vonk and Sijpesteijn, 1973). Since all five organisms investigated by Vonk and Sijpesteijn (1973) were able to methylate mercury, these researchers concluded that a slight capacity to methylate murcury may be a rather common property of aerobic bacteria. Since fungi generally do not contribute significantly to the microbial activity in lake sediments, their role in methylation may be more important in soil.

In addition to the type and number of microorganisms, microbial growth rate has also been found to influence methylation rates of mercury (Langley, 1971; Bisogni and Lawrence, 1973). Bisogni and Lawrence (1973) have reported that with doubling of net specific anaerobic growth rates (from 1/24 per day to 1/12 per day) the net specific methylation rate increased by a factor of approximately 3. A similar doubling of the aerobic growth rate caused approximately a two fold increase in the methylation rates.

(iii) Adsorption and Chelation of Metals: The adsorption of elemental contaminants to inorganic and organic constituents of water and soil systems is well documented (Dall Aglio, 1970; Hinkel and Learned, 1969; Lagerwerff, 1972). Both adsorption and complexation of the metal ion may influence the methylation process by making a particular metal ion unavailable for methylation.

(iv) Presence of other chemicals: The presence of a number of chemicals has been shown to influence the methylation of inorganic metal ions. The effect is generally observed via one of the following mechanisms: The chemical may influence the metabolic activity of the test organisms; the chemical may react with the metal ion thereby rendering it unavailable for methylation; sometimes the chemical may be preferentially methylated. Fagerstrom and Jernelöv (1971) were able to show that methylmercury production is dependent on the biochemical availability of inorganic mercury. These investigators observed that sulfide was extremely inhibitory to the methylation of mercury. Sulfide serves as a binding agent for mercury whereby mercuric ions are chemically converted to insoluble HgS. Sulfide is formed in anaerobic benthic regions of the environment from sulfate by sulfate-reducing bacteria. Tonomura et al. (1972) confirmed the inhibitory effect of sulfide by studying the methylation of mercury by a culture of C. cochlearium in the presence of sulfate-reducing bacteria. These researchers observed very small amounts of methylmercury formation in the combined culture compared with that produced in the control.

Fleming and Alexander (1972), while studying the alkylation of selenite, observed that dimethylselenide formation increased with increasing sulfate concentrations. Cox and Alexander (1973) have studied the effect of phosphate and other anions on the formation of alkylarsines. Their studies with pure cultures of Candida humicola indicated that phosphate was able to inhibit the synthesis of trimethylarsine from arsenite, arsenate and monomethylarsenate but not from dimethylarsinate. High antimonate concentrations also depressed the rate of conversion of arsenate to trimethylarsine, but nitrate was without effect.

The presence of biodegradable organic matter in the reaction medium has generally been shown to enhance the rates of methylation. Bishop and Kirsch (1972) observed a stimulation of methylation rates in anaerobic systems when organic nutrients, e.g., glucose-glutamic acid and acetate, were added to the medium. Wood et al. (1968) have suggested that nutrient enrichment of methylation systems (for example by the addition of sewage) would increase methylation rates by increasing the numbers of bacteria capable of synthesizing alkylcobalamines (a co-factor for methylation).

(v) Physical parameters such as pH, temperature and redox potential of the test medium: The pH of the methylating system could affect the rates of methylation of inorganic metal either by affecting the microbial enzyme system responsible for this transformation, by affecting the responsible organisms or by affecting the distribution and availability of the proper species of metal ion for methylation. Jernelöv _et al_. (1972) have reported that the pH optimum for methylation of mercury either under laboratory or natural conditions is 4.5.

Several investigators (Langley, 1971; Bishop and Kirsch, 1972) have reported that the methylation process was dependent on the temperature of the reaction medium. Bisogni and Lawrence (1973) found that temperature (range 10-30°C) significantly affects the aerobic or anaerobic methylation reaction if a constant growth rate of microorganisms is maintained. These authors have suggested that the effect of temperature on methylation observed by Langley (1971), and Bishop and Kirsch (1972) may most likely be due to temperature related changes in microbial growth rate which also affected the methylation process (see section on Microbial Activity, p. 406.

A relationship between the conversion of mercury compounds and redox potential has been studied by Tonomura *et al*. (1972). They found that methylmercury is formed by *Clostridium cochlearium* at about +50 mv. If the redox potential is lowered to -200 mv, sulfate reducing bacteria become predominant, and produce large amounts of sulfide. Mercuric ions chemically react with sulfide and are not available for methylation. These researchers failed to observe methylation under aerobic conditions also (redox potential approximately 300 mv). Vonk and Sijpesteijn (1973), and Bisogni and Lawrence (1973) were, however, able to obtain good methylation rates under aerobic conditions. Whether anaerobic or aerobic methylation plays a dominant role in methylation of mercury under natural conditions is unclear.

b. Factors Affecting Degradation of Organometallic Compounds

Only a few reports are available concerning the effect of various environmental and nutritional parameters on the degradation of organometallic compounds. The biodegradation mechanisms of organometallic compounds and purely organic compounds are somewhat similar except that degradation of organometallic compounds may give rise to an inorganic metal in addition to other chemical metabolites and end products. In view of the similarity between the biodegradation mechanisms of organometallic compounds and that of other organic chemicals, it appears likely that important variables pertinent to their biodegradation will be similar. These may include types of microorganisms, mineral salt composition, test chemical concentrations, supplementary nutrients and physical parameters such as pH, temperature, light, etc. The stimulation of the breakdown of monosodium methylarsonate (MSMA) due to the presence of supplemental organic carbon source was reported by Von Endt et al. (1968). These researchers observed that soil isolates released much greater amounts of $^{14}CO_2$ from ^{14}C-MSMA when yeast extract was added to the culture medium. Woolson and Kearney (1973) have reported that soil which had received cacodylic acid previously and presumably had an adapted microbial population, metabolized cacodylic acid more readily than fresh soil. These findings suggested that microbial adaptation could significantly affect the biodegradation of chemical compounds in the environment.

2. General Discussion of the Test Methods Used for Determining Environmental Transformation of Organometallic and Elemental Contaminants

Toxic heavy metals can enter the environment either in organometallic, inorganic or elemental form. Organometallic compounds such as phenylmercuric acetate, upon degradation, yield inorganic heavy metals which may be more or less toxic than the organic compound forms. The resulting heavy metals may be environmentally transformed via the routes previously shown in the biological cycle for each metal. The fate of organometallic compounds has generally been determined by similar methods as those employed for determination of environmental persistence of an organic compound. The more commonly used test methods have been shake culture test with mixed and pure cultures, and ecosystem studies. These methods have been evaluated in section III., p. 179. An inherent problem in studying the fate of organometallic compounds by any laboratory test method will be the accumulation of toxic heavy metal with degradation. The resulting heavy metal may inhibit further breakdown of organometallic compounds. In contrast, in natural aquatic environments, the inorganic toxic metal formed is continuously removed by dilution.

The possibility of methylation-demethylation interconversion of metallic compounds sets up a dynamic system of reversible reactions which results in steady state concentrations of various metallic and methylated forms in the environment. The disturbances produced in the steady state concentrations caused by the introduction of metal into the natural ecosystem as a result of man's activities will affect the natural equilibrium which will, in turn, affect the concentrations of toxic intermediates. In assessing the environmental fate of toxic heavy metals, therefore, what needs to be answered is not only whether a particular metal can be methylated but also if the kinetics of the process will allow significant levels of the methylated form to build up.

The test methods used thus far have succeeded in answering only part of the kinetic question, e.g., can a particular metal be methylated. The techniques used for these studies have included microbial reactors, shake culture studies with pure and mixed cultures of microorganisms, and microecosystems. The aerobic and anaerobic microbial reactions constructed by Bisogni and Lawrence (1973) appear to be more suitable for investigational use. They have used these reactors for studying the effect of various environmental conditions on microbial methylation of mercury, where a careful control of incubation conditions is desirable. More commonly used are simpler tests in which investigators have incubated pure or mixed cultures of microorganisms with inorganic forms of a metal under aerobic and/or anaerobic conditions and have identified the methylated product formed (Vonk and Sijpesteijn, 1973; Jensen and Jernelöv, 1969; Fleming and Alexander, 1972; McBride and Wolf, 1971).

Mixed cultures may sometimes give erroneous results since they may be expected to contain microorganisms responsible for both methylation and demethylation of the test heavy metal. Depending upon the conditions chosen for the test, their activities could be affected and may influence the results of the test. Natural communities, however, may be preferred over pure culture of microorganisms, since it is unlikely that any single microorganisms can methylate all the metals. Vonk and Sijpesteijn (1973) observed that all the five aerobic bacteria investigated by them were able to methylate mercury. From this they concluded that a slight capacity to methylate mercury may be a rather common property of aerobic bacteria. The use of aquatic microecosystems has become an effective tool for studying biotransformation of metals (Isensee et al., 1973; Schuth et al., 1974). The ecosystem allows the investigator to examine the interconversions of metallic compounds in a somewhat dynamic system. Since a large number of metal transformations and transport reactions are expected to take place in the model ecosystem due to the presence of a complete food chain, a kinetic study of the metal transformation is also feasible. The information obtained could be more easily extrapolated to the natural environment to assess if biological transformation of any metal would result in accumulation of toxic intermediates in significant concentrations in the environment. There can, however, always be doubt that the kinetic rate of each transformation reaction is the same in the model ecosystem as that in the environment. Since the accumulation of substantial quantities of any toxic intermediate is so much dependent on the rate of each reaction, a change in the kinetic rate of one single reaction in the model ecosystem could yield different results than what will take place in the actual environment.

Numerous reports have become available from which it is clear that mercury can be methylated either by enzymatic (microbial) or chemical mechanisms (Jensen and Jernelöv, 1969; Wood et al., 1968; Imura et al., 1971; Bertilisson and Neujahf, 1971). Whether these reactions occur to a significant extent in the environment has yet to be determined. Spangler et al. (1973b) have stated "the inability to find even traces of methylmercury in most sediments taken from areas highly polluted with inorganic mercury leads one to question whether significant methylation occurs in sediment under environmental conditions or whether the turnover rate of any methylmercury formed is such that significant concentrations do not accumulate." These investigators (Spangler et al., 1973b) have shown that a large number of microorganisms occurring in the lake sediment possess the ability to aerobically degrade methylmercury. These species may be important in suppressing the methylmercury content of the sediment. Similarly, species of microorganisms which degrade methylated forms of arsenic have also been found to be present in the environment (Von Endt et al., 1968).

In spite of the fact that a few researchers have failed to detect methylmercury in the lake sediment, it has generally been assumed that the methylmercury found in fish is the result of formation before intake beginning with methylation of inorganic mercury in sediments. Wood (1973) has pointed out that measurement of steady state concentrations need not reflect the rate of synthesis of methylmercury. He further states that the rate of synthesis of methylmercury does not have to be very rapid in sediments for fish to accumulate dangerous levels of it; when the rate at which methylmercury is produced, then released into the water and taken up by fish exceeds the rate of metabolism of methylmercury in fish, then methylmercury will accumulate in fish (Wood, 1972). The kinetics of the process is more important and, therefore, what should be measured according to Wood (1973) is the concentration of total mercury in the sediment and the rate of methylmercury uptake in fish. However, such measurements still would not distinguish whether methylmercury was taken up by or formed in the fish. This literature review has revealed no experimental studies in which measurement of kinetics of methylation has been attempted.

3. Correlation Between Laboratory and Field Results

Very few field studies have been carried out to determine the fate of heavy metals and the results are complex and difficult to interpret. In one field study the fate of mercury was investigated in artificial stream systems (Kania _et_ _al_., 1973). However, the researchers analyzed only for total mercury rather than determining the forms of mercury present.

Braman and Foreback (1973) have analyzed the methylated forms of arsenic in the environment. They have reported that dimethylarsinic acid was the major form of arsenic in the environment. Methylarsonic acid, although found, was present in much smaller concentrations. Laboratory studies have shown that microorganisms can methylate arsenic and that both dimethylarsinic acid and methylarsonic acid may be intermediates in the arsenic methylation sequence (McBride and Wolfe, 1971). In view of the fact that dimethylarsinic acid may be resistant to oxidation, and if it is reduced by microorganisms to dimethylarsine it will be readily oxidized back to dimethylarsinic acid, this form of arsenic may predominate. The actual situation is more complex than this since both dimethyarsinic acid and methylarsonic acid are added in the environment in the form of pesticides and, therefore, one will expect to find these forms in the environment. The estimated U.S. production for arsenic pesticides indicates that methylarsonic acid is produced in much larger quantity than dimethylarsinic acid [for example, in 1971, 70 million pounds of disodium and monosodium methylarsonate was produced, whereas only 2 million pounds of sodium cacodylate (sodium salt of dimethylarsinic acid) was produced, EPA Technical Report TS-00-72-04]. If no transformation of these compounds occurred in the environment, one would expect to find methylarsonic acid as the major form of arsenic in the environment. The fact that dimethylarsinic acid was the predominant form as shown by the studies of Braman and Foreback (1973), can be interpreted to mean that methylation of methylarsonic acid was occurring in the environment and was responsible for resulting in the accumulation of dimethylarsinic acid. McBride and Wolfe (1971) were able to show transformation of methylarsonic acid to dimethylarsinic acid in their laboratory studies with cell-free extracts of bacterium *Methanobacterium*.

F. Cost Analysis

The cost estimates given below are for evaluation of environmental fate of at least 20 compounds (elemental contaminants or organometallics). Since the analytical equipment cost for determining the fate of toxic metals or their organic forms is extremely high, cost estimates for studying the fate of one compound alone will be enormously high and perhaps misleading.

Table 43. Cost Estimates for Evaluation of Environmental Fate of Elemental Contaminants and Organometallics. The Total Cost Indicated is for Studying 20 Compounds.

Test Method	**Analytical Method**	Equipment needed and its cost	Equipment for the test	Chemicals glassware and misc. supplies	Labor cost @ $60/day	Overhead 125%	Total cost	Cost/Compound
Batch culture studies with mixed and pure cultures of microorganisms	Gas chromatography	Gas chromatograph - Mass spectrometer $100,000 (Depreciate over 10 year period)	1125	200	2700	3375	7400	370
Batch culture studies with mixed and pure cultures of microorganisms	Radioactivity (It is desirable to use label in the metal if organometallic compound is being investigated.	Scintillation counter $10,000	150	14,000 (includes custom made labelled material)	1800	2250	18200	910
Aerobic and anaerobic microbial reactors	Gas chromatography	Gas chromatograph - Mass Spectrometer $100,000	1350	500	3600	4500	9950	498
Model eco-system	Radioactivity	Scintillation counter $10,000	205	14,000 (includes custom made labelled material)	3600	4500	22305	1115
Cell-free extract studies	Gas chromatography	Gas chromatograph - Mass spectrometer $100,000; Ultrasonifier $500	900	200	2700	3375	7175	360
Cell-free extract studies	Radioactivity	Scintillation counter $10,000; Ultrasonifier $500	115	14,000 (includes custom made labelled material)	1800	2250	18165	908

VII. ENVIRONMENTAL DEGRADATION OF SYNTHETIC POLYMERS

A. Introduction

Stemming from crude attempts to mimic such natural products as wood, rubber, and cotton; the synthetic plastics, elastomers, and fabrics have characteristics which in many ways seem to improve rather than imitate their natural counterparts. One of these characteristics and one of the chief reasons for the versatility and commercial success of synthetic polymers is their durability or, from an environmental point of view, their persistence. The literature is rife with references to the recalcitrance of most synthetic polymers (Alexander, 1973a), to the fact that most do not degrade at "practical rates" (Potts et al., 1972). However, it would seem an error to substitute "inactive" for "recalcitrant". Degradation, if it is at all possible, is a function of time. What may be impractically slow degradation in terms of present solid waste disposal may be practical over decades of environmental exposure. On an evolutionary scale, the problem is even more evident. It may have taken millions of years for a fungus to develop that could metabolize lignin, but as the amount in the environment increase, such fungi did evolve (Nickerson, 1971). This is not to say that an attempt should or could be made to predict the fate of synthetic polymers over million year periods, but rather that the assumption that a potentially hazardous compound can be dismissed from consideration because it is polymerized may not be entirely valid. References to the burgeoning production of synthetic polymers are abundant (Alexander, 1973a; Titus, 1973; Scott, 1970). Although such polymers are usually in bulk form and thus might be expected

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to be as environmentally immobile as they are stable, their potential ubiquity is indicated by the plastic particles found floating on the surface of the Saragasso Sea at an average concentration of 290g(3500 pieces)/sq. km. (Carpenter and Smith, 1972). Further studies by Carpenter and co-workers (1972) and Colten and co-workers (1974) demonstrate that aquatic pollution with these polymers is becoming wide-spread.

The degradability of these polymers on prolonged exposure to the various conditions likely to be encountered in the environment is difficult to predict. Such predictions would best be made on a detailed understanding of the mechanisms by which polymers degrade or persist. However, up until the relatively recent interest in degradable plastics (Anon., 1972; Anon., 1971 a and b; Rodriguez, 1971), techniques used to measure the degradability of synthetic polymers have concentrated on changes in physical characteristics which might effect their utility rather than on an analysis of molecular alteration (Wessel, 1964). This is evident by the predominence of such techniques described below (see Analytical Techniques, p. 431). While some common tests may well indicate whether a polymer will degrade in the test system, they in no way illuminate the mechanism of degradation or recalcitrance. Thus, an attempt will also be made to describe the various exposure or analytical techniques that are available for determining molecular alteration, even though these are not frequently employed.

B. Techniques for Determining Degradation

1. Biological Test Methods

In designing an experimental procedure for determining the degradation of a polymeric substance, a variety of methods are available and might be classified in a number of ways, e.g., by media, duration, type of attack, etc. However, the most pragmatic classification for the design of an experimental approach is a modification of that proposed by Walchli (1968) based on the purpose of the test method. Accordingly, techniques for determining degradability can be designated as screening tests, end-use tests, or field tests. Screening tests are designed to determine if a specific factor, such as a microorganism, will modify a particular polymer. This type of test is conducted under carefully controlled laboratory conditions with as few variables as possible. Because many of these tests must be conducted in order to produce meaningful results, the test must be simple, rapid, inexpensive, and yield reproducible results.

Opposite the screening test is the field test. This test is designed to determine if a specific polymer formulation will degrade under a given set of field conditions for a prescribed period of time. In this case, while various parameters may be carefully monitored, they cannot be controlled. Consequently, interpretation may be difficult and results not readily reproducible. The test will usually require a considerable period of time (i.e., the proposed or expected service time of the polymeric component), and meaningful interpretation may require sophisticated equipment and highly trained personnel.

Between these two methods of testing is the end-use test. This type of test involves a varying amount of effort to simulate in the laboratory the conditions to which a polymer might be exposed in normal use or disposal. In such a test, certain factors affecting degradation may be experimentally altered in such a way as to make the test more simple, less expensive, or more easily interpreted, while doing minimal damage to the validity of the results. For example, shortening the duration of exposure to microbial attack may be off-set by elevating to a modest degree the ambient temperature. As long as such balancing is kept within reason, end use tests may yield accurate information. However, because of the many interactions involved under actual field conditions, the design and interpretation of end-use tests can be precarious.

a. Screening Tests

(i) Pure Culture Test on Agar - The pure culture test on agar may be described as a screening test designed to measure the ability of a specific microorganism to degrade a specific polymer. The test is popular not only to determine the degradability of synthetic polymers but also to evaluate the efficacy of protective agents on natural polymers (White and Siu, 1947). Although a number of variations are possible, the technique outlined for fungi by Rogers and Kaplan (1971) is exemplary:

(a) An inoculum is prepared of one or more strains of a single organism by culturing the organism on a suitable nutrient agar until a good spore crop is produced. The spores are washed off and used as the inoculum.

(b) Depending on the type of evaluation to be used, samples of the polymeric material are prepared and sterilized in ethylene oxide.

(c) Sterilized samples are then placed in the previously inoculated agar.

(d) The sample is then incubated for varying periods of time, depending on the type of testing to be performed. Rogers and Kaplan (1971), in testing for changes in physical properties, incubated for 42 and 84 days. However, the method is equally adaptable to 22-74 hour incubations if manometric techniques are employed (Mandels and Siu, 1950).

Although this method is widely employed, it can be considered only a crude measurement of the degradability of the test compound in the environment.

(ii) Mixed Culture on Agar - - As the name implies, this test is quite similar to the previous test except that a "representative" combination of test organisms is used (Awao, _et al_., 1971). As such, this test attempts to approximate more closely the actual conditions to which a material may be exposed in the environment. The procedure for this test has been described in some detail in ASTM D1924-70 (1970) but will, because of its extensive use, be briefly summarized here.

The organisms generally used are:

	ATCC No.*	QM No.**
Aspergillus niger	9642	386
Penicillium funiculosam	9644	391
Chaetomium globosum	6205	459
Trichoderma sp.	9645	365
Pullularia pullulans	9345	279c

* American Type Culture Collection
** Quartermaster Culture Collection

Substitutions of fungi can be made when mandated by specific test requirements. These cultures are maintained separately under favorable conditions. For testing, separate spore suspensions of each species are prepared to concentrations of $10^6 \pm 10^5$ spores/ml and verified by cell count. The separate spore suspensions are then mixed in equal volumes and serve as the inoculum. Inoculation is accomplished by spraying the spore suspension onto the appropriate surface. The carbon source may be either the test specimen or a piece of filter paper, growth on the latter being used as a viability control. The specimens are incubated for 21 days at 28-30°C and 85% relative humidity (ASTM, D1924, 1970).

This technique has been used frequently without significant modification (e.g., Darby and Kaplan, 1968; Potts et al., 1972), with and without attempts to sterilize the test sample (Berk and Teitell, 1951; Hazeu, 1967; resp.). The only appreciable modification of this procedure is the use of liquid rather than solid media, and standardization of cell number by naphelometry rather than by a counting chamber (Sharpe and Woodrow, 1971; Pankhurst and Davies, 1968).

(iii) Humidity Cabinet Test - This test simply involves inoculating the test specimen with the desired fungi and placing it in a cabinet at fixed humidity and temperature (Wessel, 1964). The basic purpose of this test is to allow growth at optimum temperature and humidity without introducing a complex soil system. Cooney and coworkers (1973) used this test on irradiated and non-irradiated samples to evaluate the effects of irradiation on biodegradability.

b. End-Use Tests: It is often difficult to distinguish clearly between screening tests and end-use tests. Prolonged exposure to mixed cultures as discussed above might in some cases serve as end-use tests. However, such fine distinctions may not be worth maintaining. For the purpose of this review, end-use testing may be characterized by the presence of a laboratory system of organisms and/or media so complex as to preclude complete and quantitative description.

(1) Soil Simulation Tests - Soil simulation tests have been used to assess the degradability of both natural and synthetic polymers (Blake and Kitchin, 1949: Walchli, 1968, Potts _et_ _al_., 1972). The samples to be tested are placed in a relatively small amount of soil and given water and air. Usually, more than a single type of soil is desirable and the pH may be adjusted. After a given period of time, the samples are removed and tested. Exposure to sterilized soil samples usually serves as the control. Similarly, exposure of the test sample to a paste of garden soil, mushroom compost, and diatomecous earth has been used to assess the degradability of both natural and synthetic textiles (Lloyd, 1955; Pankhurst and Davies, 1968).

(ii) Aquatic Submersion Test - This test is the aquatic counterpart of the soil simulation test. Although the test as designed by Muraoka (1966) specified sea water, it is equally suitable to fresh or brackish water. The samples are exposed to sterile water, sterile water inoculated with microorganisms, and sediment. The specimens may be exposed in both stressed and unstessed states at a constant 15°C for twenty-one months. In all cases, measurements are made of the pH and oxidation-reduction potentials of the water and sediment.

c. Field Tests: The technique of field exposure allows for little experimental control, although many of the physical parameters can be monitored. The emphasis is usually on the analytical procedures outlined in the latter part of this section (p. 431). In spite of the lack of experimental control, these tests remain perhaps the most definitive source of the actual environmental degradation of a polymer, although the mechanisms of such alterations can seldom be inferred. These tests fall into three basic categories by media: above-surface exposure, soil burial, and aquatic submersion.

(i) Above-Surface Exposure - Above-surface exposure implies little more than subjecting the polymer to weathering. Such items as polymeric coatings and structural components might commonly be tested in this fashion. Because any details of the exposure procedure and experimental duration are defined by the actual use conditions, no general procedure can be outlined (Walchi, 1968).

(ii) Soil Burial Test - This test is the most common and well established of the terrestrial field tests. It has been used both on natural and synthetic polymers for many years and still enjoys wide popularity (White and Siu, 1947; Potts et al., 1972; Titus, 1973). Depending on the purpose of the test, the polymer is buried in varying depths and type of soil. If non-microbial attack is expected to interfere with the results, appropriate decoys can be employed such as wood cases for termites (Farkas-Imrik, 1967). Commonly, such parameters as temperature and water content are monitored. The physical dimensions of the exposed sample are usually dependent on the type of analytical procedure to be employed (Hueck and von der Toorn, 1965). In most cases, the only control is an unburied sample, although controls to test soil activity - e.g., rate of cellulose decomposition - have been recommended (Jones, 1968).

(iii) Aquatic Submersion - Aquatic submersion techniques may be considered simply as the aquatic counterpart of the previous techniques in that aquatic testing most often involves both exposure to water and sediment (Snoke, 1957). However, aquatic systems do present somewhat unique problems in terms of exposure periods, proper control, and retrieval. The most extensive methodology is that described by Muraoka (1969). A large number of separate specimens can be attached to a single submersible unit constructed so that the material is exposed both above and below the sediment. In order to facilitate comparisons between different test sites, several parameters must be measured including depth, temperature, oxygen concentration, salinity, pH, hydrostatic pressure, current, and nature of sediment. When possible, visual observation is desirable, either directly or by remote camera. If boring or fouling organisms must be considered, various protective or bait devices can be attached to the test samples (Connolly, 1963). Because a considerable time lag may be anticipated between retrieval and laboratory analysis, care must be taken to maintain as closely as possible the exposure environment during transport. This is especially critical when testing for water absorption. Although Muraoka's (1969) procedure is specifically designed for pelagic testing, littoral exposure requires about the same approach (Snoke, 1957).

2. Phyriochemical Test Methods

Polymers may be degraded by a variety of non-biological agents. In terms of exposure methodology, these agents may be classified as liquids, gases, and light. In theory, all of the methods employing these agents are less complex than biological exposures in that the experimental conditions are usually well defined. In practice, however, many of the non-biological exposure techniques require somewhat more extensive experimental apparatus than their biological counterparts.

a. Degradation by Liquids: Water is by far the most important liquid in the degradation of polymeric substances (Titus, 1973). It may potentially affect a number of polymers having hydrolysable groups causing chain cleavage, strength loss, and changes in dielectric properties. As a rule, exposure techniques consist simply of placing the polymer specimen in water at a given temperature for a specific duration and then analyzing for degradative changes (Anon., 1971; Brown and Reinhart, 1971). In addition, this type of exposure is often used as a control in determining the effect of aquatic microbial degradation as discussed in the preceeding section (Muraoka, 1966).

b. Degradation by Gases: Exposure to gaseous agents such as oxygen, ozone, and nitrogen is most often accomplished in a flow system at a constant temperature and concentration for a given period. Ozone, for instance, has been tested at 2% conc., 9g/hr., 50°C for 6 hours (Potts _et al_., 1972) and 5.5% conc., 0.51g/min., 40°C, for 5 hours (Gutfreund, 1971). This method has also been used to test the effects of $N_2O_4$and Cl_2 (Gutfreund, 1971). A modification of this method is the use of corona discharge. This involves placing the polymer sample in an evacuated tube, flooding the system with the desired gas flowing at a constant rate, and exposing the gas-polymer preparation to a given electrical change for a specific period of time. The reaction chamber, called a corona cell, consists of two non-conducting concentric tubes separated by a small (0.5cm) gap. A ground electrode is attached to the outer tube, while the inside tube is connected to a high frequency generator (Carlsson and Wiles, 1970). Although direct analogies to environmental degradation requires caution, exposure to pure gases, either with or without coronal acceleration, can be used to assess the potential atmospheric degradability.

c. Degradation by Light: Exposure to various wave lengths of light is perhaps the most critical area of physiochemical polymer degradation, and is commonly conducted both _in_ _vacuo_ and with various gases (Day and Wiles, 1972b). Depending on the nature of the polymer, experimental specimens may consists of films, powder, or solutions. Films are by far the most common type of specimen used and are prepared by dissolving the polymer in a suitable solvent, pouring the mixture into a cast (e.g., glass plate), and allowing the solvent to evaporate (Hummel, 1966). The key phase, however, is "suitable solvent." The possibility of solvation in some way effecting the original polymeric structure must be considered. With polystyrene, for instance, the quantum yield for hydrogen formation can be radically affected by the type of solvent in which the sample is dissolved (Fox, 1967). This potential solvent problem can be avoided by preparing the film from powder. A known amount of polymer powder is placed evenly on a quartz plate and then melted. The polymer surface is then covered with another quartz plate and compressed. The samples are stripped from the quartz and vacuum dried. This method of preparation does, however, involve alternate cooling in dry ice and heating to 225°C. Thus, the thermal stability of the specific polymer must be given careful consideration before this treatment is adopted (Carlsson and Wiles, 1969).

The light used to determine photodegradation can be obtained from a variety of sources (e.g., mercury vapor, xenon arc, carbon arc). Mercury vapor lamps of low pressure (2537Å), medium pressure (2200-4000Å), or high pressure (higher intensity in the 2000-4000Å region) are common with filters being used on medium and high lamps to isolate the desired wave lengths (Fox, 1967). The importance of fully characterizing the emissions from the selected light source is evident from the work of Day and Wiles (1972a). Comparing the emission curves of xenon arc with Pyrex/clear glass filters and carbon arc with clear Pyrex globe, the actual output at 3400Å is more than double with the xenon arc. After 1000 hours of exposure of a 17,500 $\overline{M}n$ polymer, the xenon arc reduced the $\overline{M}n$ to 13,800, whereas the carbon arc reduced it only to 16,900.

As with the biological techniques, physiochemical exposure may take the form of a field test. Although the details of exposure conditions may vary, it usually involves placing the polymer sample above ground (minimizing biological affects) allowing attack by all three of the above discussed degrading agents (Potts *et al*., 1972).

C. Analysis Procedures

A variety of methods have been used to assess the degradability of polymeric substances. In general, they can be divided into three distinct groups: those measuring changes in the mechanical properties of the polymer, those measuring the response of biological systems, and those measuring molecular changes. As with the methods of exposure, the type of analysis used is usually based on the type of test desired and the proposed use of the compound.

1. Changes in Mechanical Properties

The degradation of a polymeric substance might be reflected in any of a host of physical or chemical changes. Certain factors, however, can be discarded either because of difficulties in quantification or a lack of reliability. Consequently, such alterations as cracking, warpage, and mechanical erosion, which often may indicate a form of degradation, may be of little use because of the inherent difficulties in accurately describing such processes (Carpenter and Smith, 1972). Similarly, changes in size may reflect degradation but more direct factors such as absorption, leaching, or embrittlement are more readily measured and directly related (Muraoka, 1969). Even very obvious physical changes as discoloration must often be discarded in analysis because they are not necessarily closely associated with actual deterioration (Rogers and Kaplan, 1971).

Even excluding the above-mentioned factors from a quantitative determination of degradation, several physiochemical parameters for which specific techniques have been developed can be considered as analytical candidates. These include:

a. Water Absorption or Transmission
b. Electrical Properties
c. Elasticity/Embrittlement
d. Hardness
e. Tensile Strength
f. Weight Loss

a. Water Absorption or Transmission (ASTM, E96-66, 1971): The amount of water in liquid or vapor phase that a polymeric substance will transmit or retain is related to macromolecular arrangement. Thus, changes in these parameters can be used as an indicator of degradation if proper consideration is given to other possible factors influencing absorption (e.g., leaching of plasticizers). As a rule, water absorption is used for relatively thick specimens while water vapor transmission is used for thin sheets or films which might be expected to exhibit membranous properties.

Water absorption has been used to determine the response of plastics to marine exposure, although it could be adapted to other types of exposure (Muraoka, 1969). Basically, water absorption is determined by changes of weight in the specimen before and after submersion in water. Because the primary concern is with changes in the percent water absorbance by weight, two identical samples should be used. The sample should be weighed prior to exposure (W_1), immediately after submersion termination (W_2), and after conditioning at 23°C and 20% relative humidity until weight stabilizes (W_3). In marine exposure where borer attack may lead to significant weight loss, W_1 must be discarded. Percent water absorption is then $W_2-W_3/W_3 \times 100$. In cases where W_1 is not obviously invalidated, discrepancies between W_2-W_1 and $W_2 - W_3$ may indicate non-degradative weight changes such as additive leaching or evaporation.

Water vapor transmission involves sealing the test specimen over the mouth of a dish containing either water or a water absorbant substance. The entire system is weighed, then placed in various temperature/humidity systems at constant pressure. Weight gain [using desicant] or loss [using water] can then be used to measure total vapor transmission over the experimental period per unit of surface area, the accepted unit of measurement being $g/24h\ /\ m^2$ at specified experimental conditions (ASTM, E96-66, 1971).

b. Electrical Properties: Many factors, both physical and biological, can affect the electrical properties of polymeric materials. In field testing it is often difficult to distinguish between the effects of moisture and fungi, especially in that even subvisual growth of fungi can produce marked effects (Snoke, 1957; Greathouse et al., 1951). However, with proper experimental controls, the distinction can often be made and the change in various electrical properties closely correlated to polymer degradation (Muraoka, 1966).

For solid polymeric electrical insulating material, the high-voltage, low-current dry arc resistance test can serve as a useful screening test for degradation (ASTM, D495-70, 1971). This test involves connecting either a tungsten rod electrode system or stainless steel strip electrodes a given distance apart to the surface of a plastic sheet, applying a specified voltage, and observing the arc formed. As the voltage is increased, failure of the insulating material will occur in a relatively short period of time by the formation of a line of conductance and the consequent disappearance of the arc into the material. Variations in failure time may then be used in assessing degradation. Certain types of material, however, may fail in different ways, such as combustion or carbon formation. For these types of material, the test has little relevance (ASTM, D495-70, 1971).

An electrical property test more closely related to end-use rather than screening is a measurement of changes in dielectric breakdown voltage (ASTM, D149-64, 1971). This test is conducted at commercial power frequencies and can be used to measure changes in insulating ability after exposure to various degradative processes. The test basically consists of applying a given voltage or voltages to the test material over a given period of time under specified temperature and humidity. The specific conditions of the test should simulate the actual use of the material to be tested.

Dielectric breakdown is preferably determined by the physical decay or distortion of the material rather than an increase in current. The test itself can be conducted in any of these ways: a rapid voltage rise over a short period from zero voltage to breakdown voltage, a slow continuous rise in voltage for 50% breakdown to breakdown, and an even increment rise from 50% of breakdown to breakdown.

While both the arc resistance test and the breakdown voltage test measure changes in decomposition points from electrical stimulation, more subtle characteristics such as the dielectric constant can also be measured (ASTM, D150-70, 1971). This type of testing involves rather sophisticated apparatus and careful interpretation which, in essence, involves direct electrical measurement of capacitance by constructing an experimental capacitor from the test material. Changes observed in dielectric constant can be caused by physical or chemical changes, moisture absorption, and surface ionization. A more manageable and readily measured parameter may be the change in DC resistance caused by degradation. This can be determined simply by direct measurement of voltage or current under specified circumstances (ASTM, D257-66, 1971).

c. Elasticity/Embrittlement: Changes in elasticity may indicate actual alterations in the polymer. Photooxidation, for instance, of polyethylene or nylon may lead to cross-linkings which result in embrittlement (Lightbody *et al.*, 1954). However, the simple loss of plasticizer may also lead to elasticity loss without necessarily indicating any actual degradation at the molecular level (Carpenter and Smith, 1972). Two quantitative methods can be used for the measurement of elasticity, the cantilever beam technique or the vibrating reed method.

The vibrating reed method involves calculation of the elasticity modulus through experimental measurement of the resonance frequency of the sample (Hazeu and Waterman, 1965). The results obtained by this method are highly dependent on the thickness of the sample and the sensitivity is greatly diminished by samples approaching 1.0 mm.

The cantilever beam method measures the elasticity of a material by experimental determination of the force and angle of bend. Thus, the deflection angle caused by a known force can be used to indicate stiffness. The physical distortion of the test material, however, indicates both plasticity and elasticity; thus, derivation of the elasticity modulus is not possible. However, this method is suitable for measuring relatively thick specimens--so long as the length-to-thickness ratio exceeds 15 to 1--but loses accuracy as the thickness decreases to 0.5 mm (ASTM, 747-70, 1971).

d. Hardness: For thicker test specimens, hardness rather than elasticity may be a more dependable indicator of degradation. The basic procedure for testing the hardness of plastics (ASTM, D785-65, 1971) recommends a thickness of at least 0.6 cm, although the method can be adapted for testing tape samples (Pankhurst and Davies, 1968).

The basic premise in hardness testing is the hardness can be correlated to the depth of penetration of a steel sphere, pyramid, or other device under a fixed load. In practice, the index of hardness is determined by the increase in penetration from a smaller initial indenter load to a larger load. A variety of devices are available for measuring hardness by this method; e.g., Rockwell Hardness Tester (ASTM, D785-65, 1971); Durometer (Muraoka, 1969); Wallace Microhardness Tester (Pankhurst and Davies, 1968). While the scale readings with each may vary, they can be converted to indentation depth, e.g., one scale division in Rockwell Tester may equal 0.002 mm. More important in comparing results of different tests is the size of the indenting sphere and the interval of time between the minor and major strokes, both of which have a marked effect on the depth of penetration. However, in measuring deterioration, the main concern is in the difference between exposed and control samples, making conversions often unnecessary. The prime factors in experimental testing are that the samples be uniformly clean and conditioned (Pankhurst and Davies, 1968). When possible, such hardness tests should be compared to other relevant parameters such as moisture absorption (Muraoka, 1969).

e. Tensile Strength: Changes in tensile strength has long been a popular method for measuring the degradation of polymers, both natural and synthetic (White and Siu, 1947). The basic method has altered little. After exposure, the material to be tested is cleaned, dried, and conditioned. It is then placed in an appropriate testing device. This tester usually consists of two vise-like jaws, one of which separates from the other at a fixed speed with a gauge for measuring load. Commonly, changes in breaking strength are measured (Berk and Teitell, 1951). This basic method can be modified to accommodate a wide variety of samples ranging from extremely small samples of plastic (ASTM, D638-68, 1971) to nylon and polypropylene ropes with tensile strength in the thousands of pounds (Muraoka, 1969).

Detailed methods for conducting this type of test are outlined in ASTM, D638-68. As a rule, this tensile strength is often a reliable indicator of degradation (Rogers and Kaplan, 1971; Pankhurst and Davies, 1968) and correlates well with other parameters (Mandels and Siu, 1950, Potts _et_ _al_., 1972).

f. Weight Loss: When dealing with a pure polymer or when the effects of various additives can be precisely determined, weight loss can be a very reliable indicator of degradation (Hazeu, 1967; Potts _et_ _al_., 1972). The procedure involves simply the weighing of the specimen both before and after exposure using proper conditioning techniques to eliminate the effects of moisture absorption (Hitz and Zinkernagal, 1967; Mandels and Siu, 1950). When measuring biodegradability, an aseptic control may be introduced (Sharpe and Woodrow, 1971).

2. Response of Biological Systems

The various tests for correlating biological response of selected organisms to the degradation of polymeric substances all attempt to, in some way, monitor metabolic activity. This is most often done by either respirametric measurements or estimates of cell multiplication.

Perhaps the most common method is the determination of BOD. This has been used by Snoke (1957) to screen for biological degradation of a number of synthetic polymers. Water containing a known amount of oxygen and the test polymer is inoculated with liquid from an enrichment culture. Controls consist of polymers submerged in water. Incubation is carried out in darkness in a water bath at a constant temperature. At various intervals

(0,1,2,4,8 weeks) dissolved oxygen is measured. Although this test can be used as a qualitative determination of the ability of the polymer to be used as a carbon source, quantitative determinations are highly dependent on the surface area of the test sample.

A direct determination of oxygen consumed over a relatively short (48 hr.) period can be obtained using differential manometry (Mandels and Siu, 1950). In this method, two flasks, experimental and control, are connected to a manometer as diagrammed below.

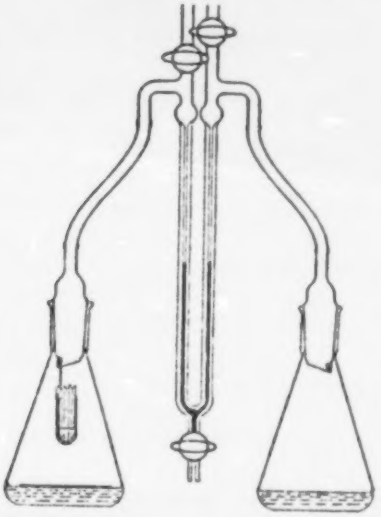

Figure 51. Design of Differential Manometer
[Mandel and Siu, 1950]
Courtesy of American Society for Microbiology

The experimental flask contains nutrient agar, the test substrate, and the inoculum. The control contains only the agar. The CO_2 released is absorbed by KOH(10%) and the oxygen consumed is calculated by displacement of the manometer fluid. Although rapid and technically uncomplicated, this test is based on the premise that initial growth rate is directly related to degradability. While this assumption may be valid for measuring the relative rate of metabolism of readily degradable compounds, its applicability to the long-range environmental stability of recalcitrant polymers is dubious.

A procedurally more complex but probably more precise direct measurement of oxygen uptake is available using the Warburg apparatus. A microbial inoculum is prepared by centrifugation. Standard Warburg flasks containing aqueous NaOH in the central chamber and the inoculum in the main chamber are used. The side arms contain either the test substance or distiller water. Thermobarometric control flasks contain only distilled water (Pankhurst and Davies, 1968).

The evolution of metabolic by-products have also been used as indices of degradation. Given that certain bacteria metabolize endogenous protein in the absence of external nutrients, the degradation of a test material may be inversely related to NH_3 evolution. This test is conducted in a non-nutritive broth containing the inoculum and test material. Test samples are omitted in the control. Ammonia determinations are made from a given volume of the broth after incubation. Protein is removed by precipitation with trichloroacetic acid and centrifugation. Nessler's reagent is added to the supernatant and optical densities determined spectrophotometrically (Sharpe and Woodrow, 1971). A similar approach has been used to determine anaerobic degradation by sulfate-reducing bacteria in sea water with measurement of hydrogen sulfide (Snoke, 1957).

An alternative to the above methods is the visual determination of bacterial growth. Using an agar plate, this type of observation can be made over a wide if somewhat inexact range of quantitative tolerances (Muraoka, 1969; Brown, 1946; Jones, 1968; Potts et al., 1972; Walchli, 1968).

Using liquid culture, however, comparatively exact estimates of microbiological growth can be obtained by either direct or indirect measurements. A viable cell count can be obtained by the surface drop method. This involves inoculating an agar plate with a fixed volume of liquid culture after exposure to the polymer. Before inoculation, the sample is diluted so that clear clones can be recognized. The number of clones established allows for direct measurement of total number of viable cells in the polymer culture sample (Pankhurst and Davies, 1968; Sharpe and Woodrow, 1971). An indirect measurement can be obtained by nephelometric determination of optical density in inoculated cultures. With this method, samples may have to be diluted as turbidity increases with growth (Hitz and Zinkernagel, 1967). Using either direct or indirect liquid culture methods, the samples must be shaken constantly to insure homogeneity of culture conditions.

3. Molecular Alteration

While changes in mechanical properties and response of biological systems may indicate that degradation is or is not occurring, these changes only provide indirect evidence for degradation. However, providing direct evidence of degradation of polymers requires a radically different approach from that of non-polymeric substances. Common analytical techniques, such as gas chromatography, TLC, and mass spectrometry, which are frequently used with lower molecular weight compounds, are not readily applied to most polymers. Instead, changes in molecular weight and/or indications of bond rearrangement or substitution are needed to satisfactorily describe polymer degradation.

Perhaps the only true indicator of polymer degradation is "backbone" cleavage and the most reliable indicator of "backbone" cleavage is a decrease in molecular weight. With most synthetic polymers, however, molecular weight is more often an average than an absolute. Two common ways of expressing this average are "weight average molecular weight" ($\overline{M}_w$) and "number average molecular weight" ($\overline{M}_n$). Values of $\overline{M}_w$ are based on techniques such as light scattering, sedimentation rate, and some viscosity readings, which determine the weights of the various molecular fractions in the polymer sample. The $\overline{M}_n$ is dependent on the number of molecules in a given mass and is usually calculated from osmotic pressure measurements. As a rule, techniques used to determine $\overline{M}_w$ are more influenced by the larger molecules in a given sample, whereas $\overline{M}_n$ determinations measure large and small molecules with equal sensitivity. Thus, in a given polymer sample, $\overline{M}_w$ will be greater than $\overline{M}_n$ unless the sample is molecularly homogeneous. As the heterogenicity increases, so will the ratio of $\overline{M}_w$ to $\overline{M}_n$. This ratio ($\overline{M}_w/\overline{M}_n$) can be used as an index of polymolecularity or changes in chain length distribution (Thomas and Kendrick, 1969).

Sedimentation rates from which $\overline{M}_w$ can be calculated are obtained by ultracentrifugation of a polymer in a solvent of lower density than the polymer. Change in refractive index of the solvent are used to monitor polymer sedimentation. However, the rate of movement is dependent not only on molecular weight but also on the shape of the molecules, with spherical molecules settling more rapidly than cyclindrical ones. While this method is frequently used to characterize protein macromolecules, it is not widely employed on synthetic polymers.

Light scattering measurements involve comparisons of the amount of light transmitted by a pure solvent compared to that transmitted by a solvent-polymer mixture. The amount of light scattered is related not only to the number of molecules present but also to their size, with large molecules scattering proportionately more light than smaller molecules. Thus, only an $\overline{M}_w$ can be derived from this method. While somewhat more popular than ultracentrifugation (Fox, 1967), this method is rarely used for molecular weight determination. However, light scattering measurement can also be used with polymer films as an index of degradation without actually determining molecular weight (ASTM, D 1003-61, 1971).

Osmotic pressure measurements are conducted by dissolving the polymer in a solvent and placing this mixture on one side of a membrane selectively permeable to the solvent but not the polymer, with pure solvent on the other side of the membrane. The osmotic pressure can be determined either by static or dynamic methods. The static method measures the fluid flux in a calibrated capillary tube connected to the polymer-solvent side of the membrane, indicating solvent influx. This method is probably the more accurate and requires less sophisticated apparatus, however, a considerable period of time is usually required for the system to reach equilibrium. The dynamic method utilizes an osmometer in which the flow of solvent is prevented by the external application of a known pressure, the primary advantage being the rapidity with which the test may be made (Golding, 1959).

Viscosity measurements to determine molecular weight is the most popular method in dealing with synthetic polymers (Carlsson and Wiles, 1969a; Day and Wiles, 1972b; Potts et al., 1972). Basically, viscosity values are obtained by passing a dilute polymer sample under a given pressure through a capillary tube and measuring the rate of flow. In practice this is done by any number of commercially available dilution viscometers. The intrinsic viscosity $[\eta]$ of a homogeneous polymer is related to molecular weight as: $[\eta] = K\overline{M}^a$. The constant "a" depends on the rigidity of the molecule, varying from 0.5 to 2.0 as rigidity increases. The constant "K" is specific for a polymer-solvent mixture. For non-homogeneous polymers, the molecular weight obtained is equal to $\overline{M}_w$ if "a" = 1 (Golding, 1959). If "a" $\neq$ 1, the molecular weight is often given simply a $\overline{M}_v$, viscosity average molecular weight (Potts _et_ _al_., 1972). The value is usually based on a standard $[\eta]/\overline{M}_v$ curve of analogous polymers of known molecular weight. Further, if both "a" and "K" are known, it is possible to obtain $\overline{M}_n$ from viscosity measurements as done by Day and Wiles (1972a) for polyethylene terephthalate:

$$[\eta] = 1.7 \times 10^{-4}\overline{M}_n^{0.83}$$

Although changes in molecular weight are vital in monitoring polymer degradation, more precise information on types and degrees of changes in bonding patterns can be obtained from standard infrared spectrophotometry (e.g., Delman _et_ _al_., 1969; Gutfreund, 1971). As with non-polymer compounds, absorption characteristics in the infrared regions are highly specific and changes in absorption patterns indicate molecular structure alterations. Samples used in spectrophotometric measurements may be prepared as films cast from solutions, pressed or melted films, microtomed samples, paraffin oil mulls, or KBr disks. The thickness of the sample depends on the absorption intensity of the polymer and may vary from 0.005 mm to several millimeters (Hummel, 1966). Polymers, of course, present highly complex spectra. In some cases, curve resolvers (e.g. DuPont 310) are necessary to resolve complex infrared envelopes (Carlsson and Wiles, 1969b). When spectra are recorded between sodium chloride discs, tetrachloroethylene has been used to preclude interference bands (Day and Wiles, 1972a).

In that degradation of polymeric substances is often restricted to the surface, an infrared technique has been developed which can determine the penetration of the attack by identifying polar groups between 0.1 and 2 μm in depth (Cooney et al., 1973). This technique is referred to as "attenuated total reflection spectroscopy." Basically, the technique involves focusing an IR beam on a polymeric film with a suitable reflective backing. The beam undergoes total internal reflection and various frequencies are diminished depending on the absorption characteristics of the polymer (Carlsson and Wiles, 1969). The absorption characteristics at varying depths are obtained by varying the reflective element and the angle of incidence of the IR beam (Carlsson and Wiles, 1971b).

Various types of thermal testing are also useful in determining degradation. Perhaps the most elementary measurement is heat of combustion (cal./g) using a bomb calorimeter. This procedure measures essentially the amount of energy in a polymer. Degradative changes such as backbone cleavage would thus be reflected in a decreased heat evolved during thermal decomposition. More detailed information may be obtained from differential thermal analysis. In this method, the polymer sample is slowly exposed to a wide temperature range and changes in heat energy are compared with that of an inactive high thermal stability control. Decomposition is indicated by the polymer's heat absorption while condensation or crystallization is indicated by heat release. Changes in the temperature at which these thermal reactions occur indicate molecular rearrangement (Gutfreund, 1971).

Depending on the nature of the polymer and the degrading agent, various other types of analytical procedures may be employed. Simple light microscopy has been used on microtomed samples of methane rubber to note mycelial penetration (Awao, et al., 1971). Similarly, electron scanning can be used to characterize either surface changes in the polymer sample (Potts et al., 1972) or growth of microorganisms (Cooney et al., 1973). The degree of crystallinity can be measured by X-ray diffraction, although this technique is not widely used to detect degradation. Chemical reactivity can be used to indicate alternatives in end groups or side groups. One method is exposing the polymer to HNO_3 and then determining the amount of residual nitrogen in the sample by the Kjeldahl test. This involves degradation of the polymer nitrogen with concentrated sulfuric acid and converting it to ammonium sulfate which can then be measured photometrically (Gutfreund, 1971). Lastly, volatile products from the degradation of polymers may also be measured. This can be done by standard mass-spectrometric and gas-chromatographic methods (Day and Wiles, 1972c). When only one volatile decomposition product is evolved, manometric techniques may also be employed (Fox, 1967).

D. Evaluation of Techniques

1. Factors Affecting Degradation

a. Biological Degradation

The design of an experimental procedure for determining the biological degradation of polymers can be divided into seven distinct phases of evaluation.

(i) Selection of Polymer Formulation
(ii) Pretreatment of Test Specimen
(iii) Selection of Degrading Organism
(iv) Choice of Media
(v) Conditions of Incubation
(vi) Duration of Exposure

(i) Selection of Polymer Formulation: Polymers are seldom used or disposed of in a pure form. Most contain a variety of non-polymeric substances - such as plasticizers, stabilizers, fillers, pigments, and lubricants - any or all of which may undergo physical, chemical and/or biological deterioration or dissipation. Such additives may lead the researcher to the erroneous conclusion that the polymer is being degraded, especially when surface growth due to the presence of a degradable plasticizer or embrittlement due to the leaching of plasticizers are used as degradation criteria. Thus, a valid attempt is often made to determine the degradation of the pure polymer (e.g., Wessel, 1964; Potts _et_ _al_., 1972). While admittedly a necessary approach, especially for screening tests, the possibility that these results may have little application to degradation of normal polymer formulations must be evaluated. Consequently, in addition to testing the pure polymer, various formulations may also require testing. One approach is to use various additives of known degradability in the same polymer. For example, Hazeu (1967) tested PVC films with three plasticizers, two of which readily degrade (di-*iso*-octyl sebacate and adipate) and one which does not (di-*iso*-octyl phthalate). By comparing results of these three formulation, the investigator can distinguish between degradation attributable to the plasticizers rather than the polymer. While Hazeu's results cannot be refuted, the method may lead to misleading conclusions if it is assumed that a material (Plasticizer) degradable in pure form is necessarily degradable in formulation. Thus, Pankhurst and Davies (1968) demonstrated that components of a PVC type may degrade separately but show no signs of doing so when compounded.

(ii) Pretreatment of Test Specimen: When exposing a polymer to degradation by a well-defined microbial colony, it is often desirable to avoid contamination with unidentified organisms by sterilizing the test specimen. However, this sterilization, itself, may cause some form of degradation (Hazeu, 1967; Pankhurst and Davies, 1968). Nevertheless, ethylene oxide sterilization in addition to phenyl mercuryborate treatment has been successfully employed (Hitz and Zinkernagal, 1967).

Sterilization with ultraviolet irradiation is an alternate approach, but possible effects that this might have on the polymer must first be ascertained (Awao et al., 1971).

(iii) Selection of Degrading Organism: In attempting to determine biological degradation of a polymer, the choise of the degrading organism can be based on any number of factors. In general screening tests, an organism might be selected for its common occurrence in nature or rapid growth rate (Berk and Teitell, 1951). In attempting to find organisms that degrade a polymer, the organisms might be isolated by soil enrichment techniques (Sharp and Eggins, 1970; Sharp and Woodrow, 1971). In still other cases, the organism may be isolated from the material to be tested (Pankhurst and Davies, 1968). Whatever the criteria, the existence of controls exposed to similar physio-chemical factors is mandatory to assess the relative importance of biological and non-biological factors. With respect to pure vs. mixed culture exposures, the pure culture technique is generally regarded as appropriate only for screening tests, even though a single bacterial species may predominate in nature (Walchi, 1968). In mixed culture techniques using fungi, the problems of microbial antagonism and the general failure to properly consider the role of bacterial attack are common difficulties (Jones, 1968).

(iv) Choice of Media: Under controlled laboratory conditions, the nutrient-salts agar recommended by ASTM D2676 and D1924 (1970) seems to have all but universal acceptance for the cultivation of both bacteria and fungi:

Potassium dihydrogen orthophosphate (KH_2PO_4)	0.7g
Potassium monohydrogen orthophosphate (K_2HPO_4)	0.7g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.7g
Ammonium nitrate (NH_4NO_3)	1.0g
Sodium chloride (NaCl)	0.005g
Ferrous sulfate ($FeXO_4 \cdot 7H_2O$)	0.002g
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	0.002g
Manganese sulfate ($MnSO_4 \cdot 7H_2O$)	0.001g
Agar	10.0g
Water	1000.0 ml

Hitz and Zinkernagel (1967) have introduced a variation on this method with the omission of the agar. The resulting liquid medium allows for nephelometric assessment of microbiological growth, thus making quantitative estimates more valid.

While the medium described above may satisfy the mineral requirements of many microorganisms, it hardly approximates normal environmental conditions. When such an approximation is desired, a number of media modifications are possible. An actual soil sample with a variety of added carbon sources, may approximate environmental conditions in the laboratory (Lloyd, 1955; Hueck and van der Toorn, 1965). Alternatively, a medium with a well defined carbon source (e.g., filter paper) may be used (Sharp and Eggins, 1970; Walchi, 1968). The screening tests which supply no external carbon source to the organisms may be used to conclude whether a polymer is readily used as a carbon source but may not indicate the potential of a growing culture to alter a specific polymer (Walchi, 1968).

(v) Conditions for Growth: For the most part, screening tests are conducted under conditions as close as possible to ideal for the test specimen. This includes factors such as optimum light, oxygen, temperature, and pH. Admittedly, such conditions may seldom be encountered in the environment and there is a potential for such tests yielding false positives in terms of environmental degradability (Wessel, 1964). In addition, erros may be made in assuming polymer biodegradation from field studies, using polymer-additive formulations in which additive decomposition rather than polymer degradation is observed (Berk and Teitell, 1951; Rogers and Kaplan, 1971).

A potential source of false negatives in terms of degradation is that all screening and end-use tests encountered in the degradation of polymers conducted in a relatively closed system. While gases may diffuse in and out, the possibility exists that toxic products of initial polymer degradation may accumulate and inhibit further degradation. Although this has been cited as a possible source of error even in field testing (Muraoka, 1969), there is no indication that such inhibition actually has occurred in any laboratory tests.

(vi) Duration of Exposure: If any major criticism is to be leveled against exposure techniques for determining biological degradation, it might justly be made on the failure of many tests to allow for sufficient time for degradation to occur. Many tests, especially those using respiratory techniques for analysis, are conducted over extremely short time periods with many using no external carbon source. Thus, a compound that is degraded at a very low rate might appear to be non-biodegradable in these short periods of exposure. However, all investigators who have utilized such methods (Mandels and Siu, 1950; Sharp and Eggins, 1970; Sharpe and Woodrow, 1971; ZoBell and Grant, 1942) recognize this factor. These tests are designed to determine if a compound is readily degradable, not if a compound has the potential to degrade or even will degrade under environmental conditions.

End-use tests and field tests, however, have somewhat higher goals with consequent longer exposure periods of months or years (Cooney et al., 1973; Muraoka, 1966 and 1969; Hueck and van der Toorn, 1965). However, the conclusions that can be drawn from such exposure periods are still somewhat limited to stating that a specific polymer does not degrade rather than that it cannot be degraded. Given that the concentration of polymeric waste is constantly rising and that it may already be quite high in certain mini-environments (e.g., land fills), the possibility of microorganisms evolving necessary enzyme systems to degrade and metabolize synthetic polymers cannot be dismissed (Nickerson, 1971).

b. Physiochemical Degradation

In describing the various techniques for the exposure of polymers to physiochemical degradation, a distinction was made between attack by liquids, gases, and light. While a useful division for discussing procedural details, this classification is rather artificial. Seldom will the environmental degradation of a polymer be caused by any single element. As in the case of polyethylene, non-biological degradation will usually involve the combined effects of light, oxygen, and water (Scott, 1970). Thus, many of the exposure techniques do not attempt to distinguish between these effects and, except in cases where basic research is being conducted into the mechanism of degradation

(Day and Wiles, 1972c), such distinctions hardly seem indicated. What is of more importance is the relevance of the exposure to conditions which are likely to be met in the environment.

For experiments designed to determine the effect of water on polymeric material, the critical factors are pH, temperature and duration. Most of the available information on hydrolysis is primarily concerned with those polymers that are known to degrade rapidly, such as hydroxypropyl cellulose and polyethylene oxide (Titus, 1973; Brown and Reinhart, 1971; Anon., 1971b). However, a variety of polymeric substances whose hydrolysis characteristics have not been well defined are known to contaminate aquatic environments (Carpenter and Smith, 1972). Although the work of Muraoka (1966 and 1969) would tend to indicate that most common polymers do not undergo appreciable hydrolysis, systematic studies would seem to be indicated.

Standard exposure methods of polymers to atmospheric gases and/or irradiation are often characterized as "accelerated weathering" tests (Delman _et_ _al_., 1969). By and large, this assumption seems justified. Photochemical degradation requires an energy level of around 80-100 kcal/mole if polymeric bonds are to be cleaved (Gutfreund, 1971). In terms of wave length, this energy can be calculated by the formula: energy in kcal/mole = $2.86 \times 10^5/\lambda$ in Å (Cooney _et_ _al_., 1973). Based on this formula and the above information on energy levels, wave lengths of 287-358 nm are the most appropriate for determining the photodegradability of synthetic polymers.

2. Internal Consistency of Results

Two interlaboratory experiments have been conducted in an attempt to standardize results and test methodologies which apply to synthetic organic polymers. Hueck and van der Toorn (1965) report the results of nine laboratories using the soil burial test with residual tensile strength as an index of degradation. Although these tests were conducted on Cu-naphthenate impregnated cotton rather than a synthetic polymer, the results are indicative of problems in quantitative comparisons of degradation testing. In general, the results for the untreated cotton controls were consistent between laboratories but the results with the impregnated specimens varied widely. Here a comparison of impregnated cotton to synthetic polymers with additives may be valid. Differences in leaching characteristics of additives or impregnates may markedly affect the rates of actual or apparent degradation, making quantitative comparisons between laboratories difficult in cases where pure polymer specimens are not used. Hazeu (1967) outlines similar results from an interlaboratory experiment using mixed culture agar plate tests on plasticized polyvinyl chloride. In this instance, laboratories were in substantial agreement when weight loss was used as an index of degradation but visual evaluation of the test specimens proved misleading. By using three different plasticizers, only two of which are biodegradable, changes in weight appeared to be due entirely to plasticizer metabolism rather than polymer degradation.

While quantitative comparisons of synthetic polymer degradation may be difficult to make because of the effects of various additives, the qualitative results are in relative agreement. In no case encountered in the literature do investigators disagree on the degradability of any pure polymer tested by the same or different test methods. Details of these results in terms of the effect of structure on degradability are discussed in section VIII., p. 461.

3. Comparison of Laboratory Results to Behavior in the Natural Environment

Because of the lack of systematic monitoring data, a satisfactory correlation between the environmental fate of synthetic organic polymers and the results of the various test methods previously discussed cannot be made. However, working with the scant data that is available, the results of tests for biological and physiochemical degradation are not in conflict with current monitoring data.

Screening, end-use, and field tests all indicate that most of the synthetic organic polymers which have enjoyed commercial success of the past two decades are extremely persistent (see Section VIII., p. 461). Monitoring data of sea surface contamination (e.g., Carpenter and Smith, 1972; Carpenter _et_ _al_., 1972; and Colten _et_ _al_., 1974), while hardly complete, tends to reinforce this conclusion. Of the three plastics that comprise 90% of the plastic municipal waste--polyethylene, 38%; polyvinyl chloride, 31%; and polystyrene, 21% (Titus, 1973)--polyethylene and polystyrene were specifically identified as common pollutants (Colten _et_ _al_., 1974). While polyvinyl chloride was not identified, it probably comprised some of the unidentified plastic material found. Similar terrestrial monitoring data have not been encountered. However, a variety of papers have dealt with the degradation of synthetic polymers under normal use (e.g., Brown, 1946; Heap and Morrell, 1968; Hueck, 1960; Lightbody _et_ _al_., 1954; Wessel, 1964). While problems such as fungal surface growth and additive leaching are common, actual polymer degradation is most often attributed to physiochemical rather than biological factors (Eggins and Mills, 1971). This is also in substantial agreement with the results of various test procedures as discussed in Section VIII D., p. 474.

E. Cost Analysis

The cost of determining the environmental degradability of synthetic organic polymers may vary widely depending upon the exposure protocols that are established and the types of analytical techniques that are employed. Because the possible approaches which could be employed are numerous, the cost analysis will be restricted to a representative series of screening, end-use, and field tests using both mechanical and molecular indices of degradation. In that the cost of exposure testing and analytical techniques are independent of each other, cost breakdowns of various procedures are given separately in Table 44. A daily labor cost including overhead for a B.S. chemist or biologist is assumed at $120/day. Because the simultaneous testing of more than one compound would most often be considerably more economical, estimates are given for both one and ten compounds.

The following outlined costs are at best only crude approximations. A variation of ± 25% could easily be expected, depending on the volume of business, local labor costs, variations in overhead, the specific compound to be tested, etc. It should be noted in Table 44, that the equipment costs do not include initial capital expenditures. It is assumed that only fully equipped laboratories would do this type of testing and that the per unit cost of such equipment would be reflected in the overhead rate. Also, the cost of running appropriate controls is included in the total estimate.

Table 44. Cost of Selected Procedures in Determining the Degradation of Synthetic Organic Polymers

Procedure	Number of Compounds	Length of Test (days)	Man-days	Labor Cost	Equipment Cost	Total Cost	Cost/ Compound
EXPOSURE TESTS							
Screening Test (Mixed Agar Plate)	1	21	0.5	$ 60	$ 10	$ 70	$ 70
	10	21	3.0	360	80	440	44
End-use Test (Soil Simulation Test)	1	60	1.0	120	20	140	140
	10	60	4.0	480	100	580	58
Field (Soil Burial Test)	1	120	5	700	50	750	750
	10	120	8	960	150	1110	111
ANALYTICAL TECHNIQUES							
Hardness or Tensile Strength	1	0.25	0.25	30	*	30	30
	10	2.5	1.5	180	*	180	18
Weight Loss Test	1	0.1	0.1	12	*	12	12
	10	0.5	0.5	60	*	60	6
Scanning Electron Micrograph	1	0.25	0.25	30	200	230	230
	10	2.5	2.5	180	1600	1780	178
Molecular Weight Determination by Sedimentation	1	0.5	0.5	60	15	75	75
	10	4.0	4.0	480	60	520	52
I.R. Spectra	1	0.2	0.2	24	*	24	24
	10	0.5	0.5	60	*	60	6

*negligible

For a complete evaluation of the environmental persistence of polymers, all levels of testing--i.e., screening, end-use, and field tests--would probably be necessary. Screening tests such as the mixed agar plate method are useful in determining whether a polymer is readily degradable under ideal conditions. However, polymers shown to be degradable under such conditions may degrade much more slowly under environmental conditions. Conversely, the lack of degradation in a screening test does not rule out the possibility of degradation over prolonged periods under natural conditions. In the same way, if positive degradation is found in a field test--e.g., soil burial--it may be desirable to determine the rates of degradation under controlled conditions.

Analytical techniques that are commonly used and quite beneficial in determining product utility--i.e., tensile strength and hardness tests--are only of tangential use in assessing environmental degradation. Other types of tests which do show molecular degradation--such as spectral analysis and scanning electron micrography--although different in price, yield much more useful and definitive information on potential environmental fate. As estimated in Table 47, the total cost of a complete series of tests - screening tests, end-use test, field test, electron scanning, and I.R. spectra - would be about $775 per compound if ten compounds were tested or $1722 per compound if one compound were tested. This testing of many commercial polymers may be justifiable on the basis of high sales volume, high unit cost, and/or cost spent in basic product research and development.

VIII. RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND ENVIRONMENTAL PERSISTENCE

Although a fair amount of information is available "about the influence of molecular structure on the toxicity to human beings of drugs and certain other chemicals, much less is known about the influence of molecular structure on the environmental persistence" (Goodman, 1973). This lack of understanding is indeed unfortunate since understanding the correlation between chemical structure and environmental persistence could be extremely useful. For example, such understanding would allow synthetic chemists to consider the environmental stability of a chemical in the early stages of commercialization. It would also allow inclusion of environmental persistence considerations when setting environmental hazard priorities for new chemicals or chemicals which have not been thoroughly tested. Similar reasoning applies to inclusion of bioaccumulation considerations in priority setting since the potential for "ecological magnification" (Kapoor et al., 1973) is closely related to the persistence both in the environment and the food chain. In addition, understanding relationships between chemical structure and environmental stability provides insight into breakdown mechanisms and pathways in the environment and provides a considerable advantage to researchers attempting to identify metabolites.

The following sections will discuss what is known about the relationship between chemical structure and persistence in the environment. The sections are categorized either by chemical group, reaction media, or mechanism of degradation.

A. Relationship of Chemical Structure and Biodegradability

Part of the reason that only a few generalizations can be drawn between chemical structure and environmental persistence is the lack of

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precise criteria for determining persistence. Two general criteria have frequently been used: the rate and extent to which a natural or enriched mixed culture degrades the compound, or the proportion of tested species, strains, or isolates which is able to use the compound for carbon and energy source (Painter, 1973a). Payne and coworkers (Payne et al., 1970; Prochazka and Payne, 1965) have concluded that "no more convincing evidence of degradability can be offered than demonstration that an organic compound can be utilized as a source of both carbon and energy by microorganisms." Horvath (1972a), however, disagrees with the growth criterion and feels that many compounds are termed "recalcitrant" (Alexander, 1965) not due to microbial infallibility but instead due to fallible biodegradability test methods which do not take into account co-metabolism (concomitant oxidation of a non-growth substrate). The importance of the co-metabolic process to environmental persistence is relatively unknown. Many studies have not examined the possibility of co-metabolic degradation and, therefore, this lack of co-metabolic evaluation should be kept in mind in the following review of chemical structure-biodegradability correlations.

The only physical property that has been found to be related to biodegradation is the water solubility of the chemical. Water-insoluble materials are frequently expected to endure longer than water-soluble materials (Alexander, 1965, 1973a). Kapoor et al. (1973) found good agreement between water solubility and their "biodegradability index" (BI=non-polar products/polar products), although the correlation between BI and the octanol-H_2O partition coefficient was very poor. Along this same line, Swisher (1970) has concluded that, with surfactants, the "chemical nature of the hydrophilic group is of only minor importance in affecting biodegradability". The reason for increased persistence with decreased water-solubility is not well understood but it is possible that the hydrophobicity can contribute to: (1) failure of the chemical to penetrate to the reaction site within the cell, (2) reduced rate of attack when biodegradation is regulated by the rate of solubilization, and (3) inaccessibility of the chemical due to adsorption or entrapment in inert material (see Alexander, 1973a).

Other parameters have also been suggested for correlation with biodegradability. For example, Torgeson (1971) suggested that "the rate at which a molecule is degraded is dependent on a number of factors such as: (1) the ease with which the molecule can penetrate the cell and reach the appropriate enzyme site, (2) the extent to which steric effects interfere with enzyme bonding, and (3) the extent to which electronic effects of molecular substituents either interfere with enzyme bonding or alter the energy required to break the critical bonds in the molecule." In order to quantify these factors, Torgeson (1971) suggested the approach used by Hansch (Gould, 1972) for correlating structure to biological activity which includes consideration of lipid solubility (octanol-water partition coefficient), electronic effects (Hammet functions), and steric hindrance (Taft steric parameter). Unfortunately, a comprehensive comparison of biodegradability to these combined factors has not been undertaken. However, Omori and Yamada (1973) have demonstrated a relationship between electronic structure and position of hydroxylation on aromatic compounds by microorganisms and, therefore, it appears that pursuit of this approach might be very fruitful.

Most comparisons of chemical structure and biodegradability are much less analytical than the approach suggested above and are usually presented as generalizations of functional groups. Painter (1973a) has suggested the following parameters which affect resistence to biodegradation: molecular size, tertiary branching, the nature, position and number of substituents in the molecule, and the presence of oxygen, nitrogen, chlorine and other atoms. Table 45 summarizes a number of studies which have examined the biodegradability of various chemicals. From these studies, some generalizations are apparent. Highly branched compounds are frequently resistant to biodegradation. This is attributed to the fact that increased substitution hinders β-oxidation, the process by which alkyl chains are usually broken down (Hammond and Alexander, 1972). This effect first became apparent when studying the biodegradability of surfactants (LAS compared to TBS) (Swisher, 1970). As a general rule, alcohols, aldehydes, acids, esters, amides, and amino acids seem to be more susceptible to microbial attack than the corresponding alkane, olefins, ketones, dicarboxylic acids, nitriles, amines, and chloroalkanes (Painter, 1973a). Ether functions are sometimes particularly resistant to microbial attack (Ryckman *et al.*, 1966). Substitution on aromatic rings can have varying effects. Groups, such as carboxyl or hydroxyl, have a tendency to increase biodegradability, while halogens and nitro groups reduce microbial attack (Alexander and Lustigman, 1966). *Meta*-disubstituted phenols and phenoxy compounds are usually more resistant than ortho- or para- isomers (Alexander and Aleem, 1971). Again, it should be emphasized that these results are dependent upon the test methods used and the criteria of biodegradation.

Table 45. Relationship Between Chemical Structure and Biodegradation

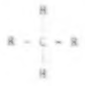
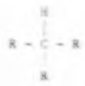
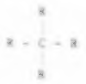
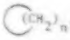
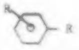
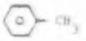
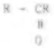
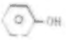
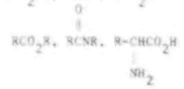
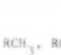
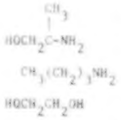
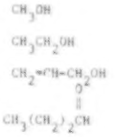
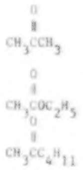
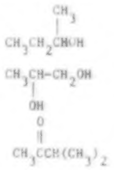
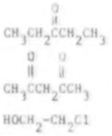
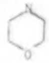
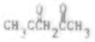
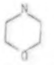
Reference	More Biodegradable (less persistent)		Less Biodegradable (more persistent)
Alexander (1965, 1973a) Swisher (1970) Painter (1973a)	Water soluble		Water insoluble
ALIPHATICS			
Mohanrao and McKinney (1962) Van Der Linden and Thijsse (1965) Iliff (1972) McKenna and Kallio (1964)	 $R - \overset{H}{\overset{\vert}{C}} - R$ with H below	 $R - \overset{H}{\overset{\vert}{C}} - R$ with R below	 $R - \overset{R}{\overset{\vert}{C}} - R$ with R below
	Aliphatic  $(CH_2)_n$, $O - C_6$, $-C_{17}$		$C_{12} - C_{16}$
	Aromatic 		
Perry and Cerniglia (1973a)	Normal alkanes, C_{10}-C_{19} Straight chain, alkanes C_{12}-C_{19}	Gases, C_2-C_4 Alkanes, C_5-C_9 Branched Alkanes, > C_{12} Alkenes, C_3-C_{11}	Branched Alkenes, Aromatics, Cycloalkanes
Ryckman et al. (1966)	$R - CH_2OH$ RCHO  $R - \underset{O}{\underset{\Vert}{C}}R$ $R - CO_2H$ 		$R - O - R$
Painter (1973a) Ludzack and Ettinger (1960)	RCH_2OH, RCHO, RCO_2H  RCO_2R, $R\overset{O}{\overset{\Vert}{C}}NR$, $R-\underset{NH_2}{\underset{\vert}{C}}HCO_2H$		 RCH_3, $RCH{=}CHR$, $R\overset{O}{\overset{\Vert}{C}}R$, $HO_2C(CR_2)_xCO_2H$, RCN, RNH_2, $R\underset{Cl}{\underset{\vert}{C}}HR$
Kaufman (1965)	$CH_3CHClCO_2H$	CH_2ClCO_2H $CH_3CCl_2CO_2H$ $CHCl_2CO_2H$ $CH_2ClCH_2CO_2H$ $CH_3CH_2CCl_2CO_2H$	CCl_3CO_2H $CHCl_2CHClCO_2H$
Ettinger (1956)	 $HOCH_2\overset{CH_3}{\overset{\vert}{C}}-NH_2$ $CH_3(CH_2)_3NH_2$ $HOCH_2CH_2OH$  CH_3OH CH_3CH_2OH $CH_2{=}CH-CH_2OH$ $CH_3(CH_2)_2\overset{O}{\overset{\Vert}{C}}H$  $CH_3\overset{O}{\overset{\Vert}{C}}CH_3$ $CH_3\overset{O}{\overset{\Vert}{C}}OC_2H_5$ $CH_3\overset{O}{\overset{\Vert}{C}}C_4H_{11}$  $CH_3CH_2\overset{CH_3}{\overset{\vert}{C}}HOH$ $CH_3\underset{OH}{\underset{\vert}{C}}H-CH_2OH$ $CH_3\overset{O}{\overset{\Vert}{C}}CH(CH_3)_2$	 $CH_3CH_2\overset{O}{\overset{\Vert}{C}}CH_2CH_3$ $CH_3\overset{O}{\overset{\Vert}{C}}CH_2\overset{O}{\overset{\Vert}{C}}CH_3$ $HOCH_2-CH_2Cl$	$(HOCH_2)_2NH$ $(HOCH_2CH_2)_3N$ $HO(CH_2CH_2-O)_2H$ $HO(CH_2CH_2-O)_3-H$ 
Hatfield (1957)	CH_3OH, $CH_3CH_2CH_2OH$, $(CH_3)_2CHOH$, $CH_3(CH_2)_3OH$, $CH_3CH_2(CH_3)CHOH$ $CH_3(CH_2)_4OH$, $(CH_3)_2CHCH_2CH_2OH$,		$(CH_3)_3CCH_2OH$, $(CH_3)_3COH$ $HO(CH_2-CH_2O)_2-H$ $HO(CH_2CH_2O)_3H$
Mills and Stack (1954)	esters, sugars, straight chain alcohols, acids, aldehydes, ketones	$HOCH_2CH_2OH$, $ClCH_2CH_2OH$, $CH_2{=}CH-CH_2OH$ $(CH_3CH_2)C{=}O$,  $CH_3\overset{O}{\overset{\Vert}{C}}CH_2\overset{O}{\overset{\Vert}{C}}CH_3$	$((CH_3)_2CH)_2O$, $(HOCH_2CH_2)NH$, $(HOCH_2CH_2)_3N$, $HO(CH_2CH_2-O)_xH$, $(CH_3CH_2)_2O$, 
Evans and David (1974)	$HOCH_2CH_2OH$	$HO(CH_2CH_2O)_2H$ $HO(CH_2CH_2O)_3H$	
Dias and Alexander (1971) Hammond and Alexander (1972)	$XCH_2-(CH_2)_n-CO_2H$ $X-CH_2-(CH_2)_n-CH_2OH$	X = Br, Cl, CH_3	$R - C(X)_n - CO_2H$ $R - C(X)_n - CH_2OH$

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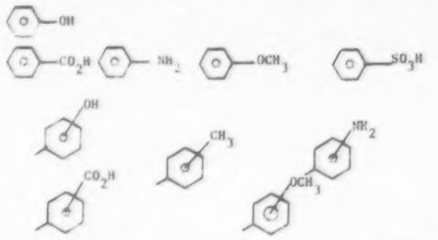
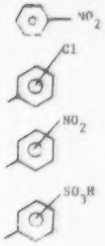
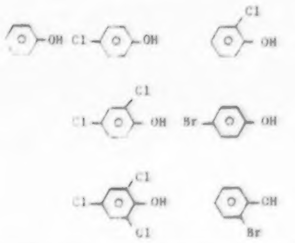
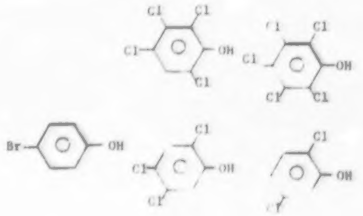
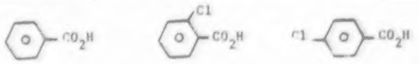
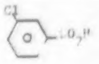
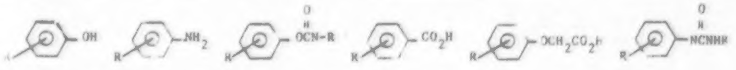
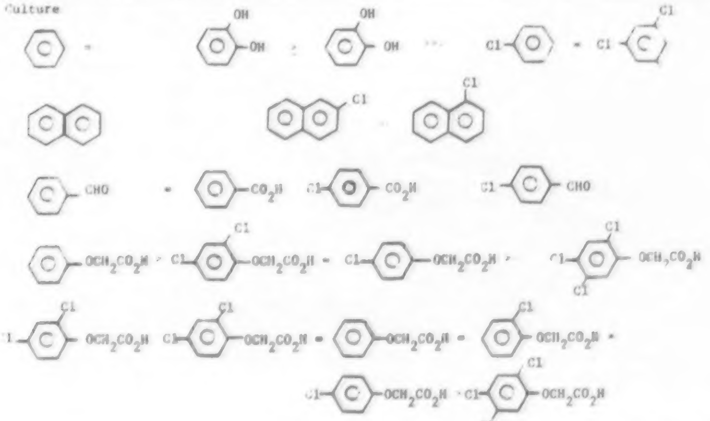
Table 45 (continued)

Reference	More Biodegradable (less persistent)	Less Biodegradable (more persistent)
AROMATICS		
Alexander and Lustigman (1966)		
Alexander and Aleem (1961)		
acRae and lexander (1965)		
Torgeson (1971)	 R = 4-Cl > 2,4-Cl > 2,4,5-Cl > 3-Cl > 3,4-Cl	
Okey and Bogan (1965)	Culture 	

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B. Atmospheric Stability of Organic Chemicals

Since the beginning of research on photochemical smog, there has been strong evidence of a correlation between hydrocarbon reactivity and chemical structure. Enough hydrocarbons have now been studied that some general trends are apparent. Most reactivities of hydrocarbons have been measured in terms of their ability to participate in the photooxidation of nitric oxide to nitrogen dioxide (e.g., Glasson and Tuesday, 1970b). However, the ranking of the hydrocarbons in terms of NO photooxidation follows the same general order as that found for hydrocarbon consumption (Altshuller and Bufalini, 1971).

For the most part, atmospheric reactivity seems to be directly proportional to the nucleophilic character of the molecule. Glasson and Tuesday (1970b) have reported the following order of reactivity: highly reactive - disubstituted internal olefins > cyclopentenes > monosubstituted internal olefins > unsubstituted internal olefins ≈ cyclohexenes ≈ tri- and tetra- alkylbenzenes ≈ diolefins > dialkylbenzene ≈ terminal olefins > more than 4C paraffins ≈ monoalkylbenzenes > propane $\geq$ 2,2-dimethylpropane ≈ benzene > ethane > methane > little reaction. Thus, the reactivity increases as the electron release into the double bond or benzene ring increases. As further evidence that the species reacting with the hydrocarbon is electrophilic in character, Glasson and Tuesday (1970b) compared the relative rates of NO oxidation of several olefins to rates for oxygen atom-olefin and ozone-olefin (in N_2) reactions. This comparison is reproduced in Table 46.

Table 46. Olefin Relative Reaction Rate Comparison (Glasson and Tuesday, 1970b)

Olefin	Relative reaction rates[a]		
	NO photoox.	O-atom[b]	O_3 in N_2[c]
Ethylene	0.48	0.04	0.21
Propylene	1.0	0.23	0.95
1-Butene	0.83	0.23	0.85
3-Methyl-1-butene	0.77	....	0.75
Isobutene	1.0	1.0	1.0
2-Methyl-1-butene	0.97	...	1.3
2-Ethyl-1-butene	0.66	...	...
trans-2-Butene	3.2	1.1	2.2
trans-2-Pentene	2.2	...	2.8
trans-3-Hexene	1.7	...	...
2-Methyl-2-butene	5.4	3.2	3.2
2-Methyl-2-pentene	4.6	...	3.2
2,3-Dimethyl-2-butene	17	4.1	5.5
2,3-Dimethyl-2-pentene	15	...	...

[a] Relative to isobutene rate for indicated reaction.
[b] Cvetanovic (1960).
[c] Wei and Cvetanovic (1963).

Reactivity trends in monosubstituted benzenes (toluene > ethylbenzene > iso-propylbenzene > tert-butylbenzene) is consistent with hyperconjugative electron release. Not all the different reactivities can be explained by electronic effects. The greater reactivity of cyclopentenes in comparison to cyclohexene can be ascribed to the greater strain energy of the five-membered ring. The greater reactivity of 1,3,5-trimethylbenzene in comparison to 1,2,4-trimethylbenzene suggests some type of steric hindrance.

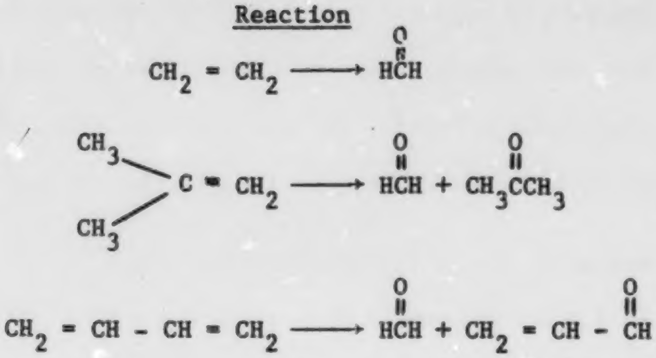
The reactivity of nucleophilic compounds can also be applied to other compounds beside hydrocarbons. For example, trichloroethylene is relatively reactive (95% loss in 6 hours irradiation) whereas tetrachloroethylene is fairly inert (Altshuller and Bufalini, 1971). This can be explained by the electron withdrawing effect of the additional chlorine atom.

When hydrocarbons are photolyzed in the presence of nitric oxide; ozone, aldehydes and ketones, carbon monoxide, organic nitrates, and small amounts of epoxides, alcohols, esters, and peroxides have been observed (Altshuller and Bufalini, 1965). The products formed from reaction at olefinic double bonds are perhaps best understood. Some examples are given in Figure 52.

Recently, the reaction of some benzilic hydrocarbons to form peroxybenzoyl nitrate (PBzN), a powerful lachrymator, has been noted (Heuss and Glasson, 1968). The ability to form PBzN, rather than rupture the benzene ring forming peroxyacetyl nitrate (PAN), is dependent upon the presence of a benzylic carbon (see Figure 53).

Reference **Reaction**

Altshuller and Bufalini (1965)

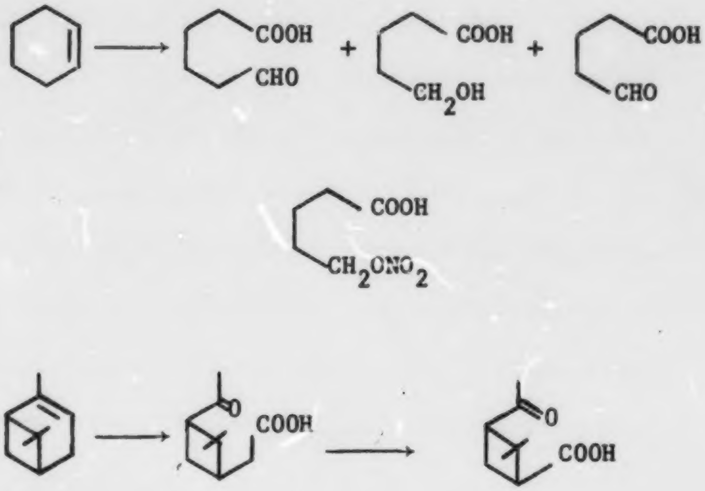


$$CH_2 = CH_2 \longrightarrow H\overset{\overset{O}{\|}}{C}H$$

$$\begin{array}{l}CH_3\\ \qquad C = CH_2\\ CH_3\end{array} \longrightarrow H\overset{\overset{O}{\|}}{C}H + CH_3\overset{\overset{O}{\|}}{C}CH_3$$

$$CH_2 = CH - CH = CH_2 \longrightarrow H\overset{\overset{O}{\|}}{C}H + CH_2 = CH - \overset{\overset{O}{\|}}{C}H$$

Wilson *et al.* (1972b)


COOH
CHO
+
COOH
CH2OH
+
COOH
CHO
COOH
CH2ONO2
O
COOH
O
COOH

Crosby and Moilanen (1974)

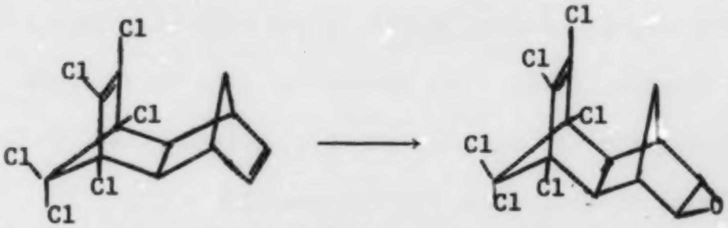

Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
O

Figure 52. Products of Atmospheric Degradation of Olefins

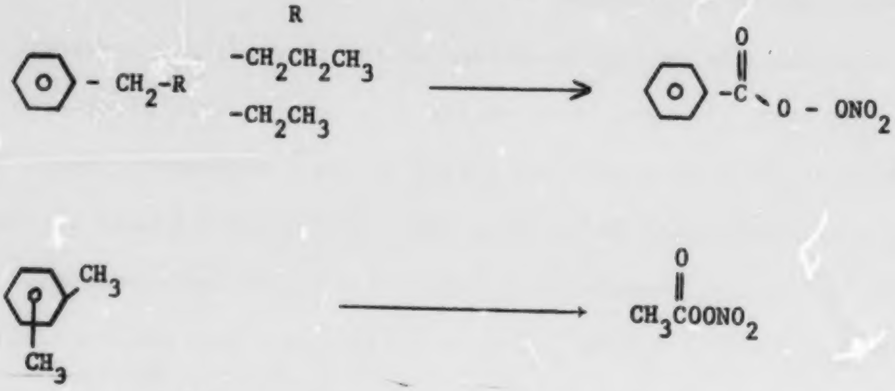

R
- CH2-R
-CH2CH2CH3
-CH2CH3
O
-C
O - ONO2
CH3
CH3
O
CH3COONO2

Figure 53. Selective Reactivity of Benzylic Hydrocarbons (Stephens, 1973)

Thus, from the above discussion, it can be seen that a great deal more is known about degradability of organic chemicals in the atmosphere. This probably is due to the more predictable nature of chemical systems as opposed to biological systems.

C. Categorization of Elements

By examining the current knowledge of the physical and chemical properties of a toxic element, it is possible to make certain predictions as to how some of these materials may behave in the environment. Such information could be helpful in deciding which of the toxic metals should be monitored in the environment. Wood (1974) has classified elements on the basis of their toxicity and their relative availability for environmental transformation, as determined by their solubility characteristics and concentrations at which they occur in the environment naturally. According to this classification, toxic elements can be considered (i) non-critical, (ii) toxic and relatively accessible, or (iii) toxic but very insoluble and very rare. The elements fit in these categories as follows:

Table 47. Classification of Elements from the Standpoint of Environmental Pollution (Wood, 1974)

Noncritical			Very toxic and relatively accessible			Toxic but very insoluble or very rare	
Na	C	F*	Be	As	Au	Ti	Ga
K	P	Li	Co	Se	Hg	Hf	La†
Mg	Fe	Rb	Ni	Te	Te	Zr	Os
Ca	S	Sr	Cu	Pd	Pb	W	Rh
H	Cl	Al	Zn	Ag	Sb	Nb	Ir
O	Br	Si	Sn	Cd	Bi	Ta	Ru
N				Pt		Re	Ba

*Some may argue with this designation, but fluoride is added to drinking water. †All the lanthanides are very insoluble and some are very rare.

The elements classified as very toxic and relatively accessible should be of major concern since they have the highest potential for environmental hazard. The relative mobility of these elements in the environment, as well as their toxicity, is somewhat dependent on their ability to undergo methylation. Wood (1974) has predicted that tin, palladium, platinum, gold and thallium will be methylated in the environment, but that lead, cadmium and zinc will not be methylated. This prediction is based on the fact that the alkyl metals of lead, cadmium, and zinc are not stable in aqueous systems and that methyl B_{12} does not transfer methyl groups to these elements.

Even if it can be predicted which metals can be methylated, it still needs to be determined whether methylation will occur in the natural environment, and if so will it result in accumulation of substantial quantities of the methylated form in the environment. The methylation process in the environment is considerably more complex than in laboratory studies, and prediction of environmental methylation rates is extremely difficult. For example, the kinetics of degradation of an alkylmetal [as shown by Spangler et al. (1973a,b) for methylmercury] will be important in determining the net quantity of the methylated form in the environment. No attempts have so far been made to formulate a methylation model which will take into account the kinetics of all the processes occurring in the environment.

In summary, some environment hazard priorities for elements can be determined by considering availability, toxicity, and solubility of the elemental forms and stability of the methylated form.

D. Structure-Degradability Relationships of Synthetic Organic Polymers

1. Biological Degradation

Very little is presently known about the effect of structure on the biological degradation of synthetic polymers. This question has received only cursory attention in the published literature with exceptions such as the work of Darby and Kaplan (1968) and the exemplary study by Potts and coworkers (1972). A listing of synthetic polymers which are subject or resistant to biological attack is presented in Table 48.

Although no absolute structure-degradability relationship can be deduced from this information, three distinct parameters affecting degradation are apparent: chain length or configuration, side group substitution, and chain bonding.

The effect of chain length on configuration is seen quite clearly in the polyethylenes. Structurally, the polyethylenes are closely allied to the natural paraffins, having the molecular formula $CH_3-(CH_2)_n-CH_3$. It has long been recognized that the lower molecular weight (smaller chain length) polyethylenes are susceptible to biological degradation while the higher molecular weight compounds are quite resistant (Lightbody *et al.*, 1954). Recent tests indicate that the straight chain polyethylenes below 500 MW can be utilized by microorganisms but that comparable sized molecules with methyl branches on every fourth carbon are resistant to attack (Potts *et al.*, 1972), suggesting a side chain effect. Haines and Alexander (1974) have shown that normal alkanes containing up to 44 carbon atoms were metabolized by microorganisms.

The effect of the side group is also evident in both the cellulose esters and ethers. The cellulose derivatives have the general formula:

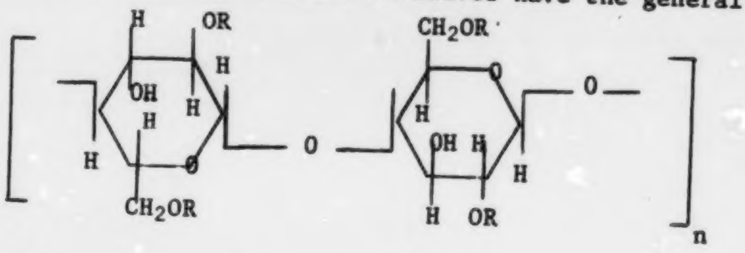
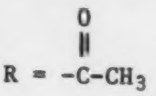


Table 48. The Biodegradability of Various Synthetic Organic Polymers (Alexander, 1973a; Brown, 1946; Greathouse *et al.*, 1951; Lightbody *et al.*, 1954; Titus, 1973; and Wessel, 1964).

Polymer	Degradable
Acrylics	
Polymethyl methacrylate	No
Polyacrylonitrile	No
Acrylonitrile-vinyl chloride copolymer	No
Cellulose	
Cellulose acetate (low acetylation)	Yes
Cellulose acetate (high acetylation)	No
Cellulose acetate-butyrate	No
Cellulose acetate-propionate	No
Cellulose nitrate	Yes
Ethyl cellulose	No
Hydroxypropyl cellulose	Yes
Rayons	
Acetate rayon	No
Cuprammonium rayon	Yes
Viscous rayon	Yes
Polyethylenes	
Polyethylene (low-~500-M.W.)	Yes
Polyethylene (high M.W.)	No
Polytetrafluoroethylene	No
Polymonochlorotrifluoroethylene	No
Polypropylene	No
Polyisobutylene	No
Vinyls and Vinylidenes	
Polyvinyl chloride	No
Polyvinyl alcohol	No
Polyvinyl butyral	No
Polyvinyl acetate	Yes
Polyvinyl chloride acetate	No
Polyvinylidene chloride	No
Polyvinylidene fluoride	No
Phenol-formaldehydes	
Phenol formaldehyde	No
Phenol-analine formaldehyde	Yes
Resorcinol-formaldehyde	No
Urea-formaldehyde	No
Melamine-formaldehyde	Yes
Polyamides	
Nylon	No
Polyurethanes	
Polyether linked	Slight
Polyester linked	Yes
Aliphatic Polyesters	
Epsilone-caprolactine	Yes
Polytetramethylene succinate	Yes
Polyethylene adipate	Yes
Aromatic Polyesters	
Ethylene glycol terephthalate	No
Chlorinated Polyethers	No
Polycarbonates	No
Epoxides	No
Siloxanes	No
Alkyd resins	Yes

In the natural polymer cellulose, the R-groups are hydrogens. In cellulose acetate - a cellulose ester,  $R = -\overset{\overset{O}{\|}}{C}-CH_3$ -, microbial resistance is imparted only by a high degree of acetyl substitution (Lightbody et al., 1954, Alexander, 1973a). The nitrate group, however, does not apparently protect the molecule from biological attack regardless of the degree of substitution.

The polyurethanes exemplify the effect of chain bonding on the degradability of polymers. Darby and Kaplan (1968) tested a variety of polyether and polyester linked polyurethanes. The polyether polyurethanes showed varying degrees of biodegradability depending on the number of side chains in the diol moiety and the degree of methyl substitution in the polymer chain. All of the polyester polyurethanes, however, were highly degradable.

Although the general influences of chain length, chain linkage, and side group substitution are apparent in the above examples, absolute structure degradability relationships cannot be drawn. As Darby and Kaplan (1968) indicate, each polymer or series of polymers must be evaluated on the basis of experimental evidence. For example, while the polyester linkages in conjunction with aliphatic groups seem conducive to biological deterioration, degradation is blocked by extensive methylation of the polymer chain as in polymethylmethacrylate. Thus, while specific aspects of polymer structure can be correlated to degradability, the predictive value of these correlations is not very quantitative.

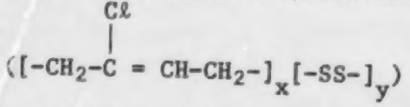
2. Physiochemical Degradation

The roles of various physical and chemical factors which affect the degradation of synthetic organic polymers have received far more extensive characterization on the molecular level than the biological factors. Although a given physiochemical agent seldom acts alone in polymer degradation, five basic factors may be isolated for initial consideration: temperature, water, oxygen, ozone, and sunlight.

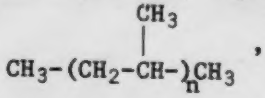
The effect of temperature in the environmental range has received the least attention of the above items because it cannot be divorced from a consideration of at least one of the other factors. It seems obvious that as the temperature increases the effects of water, oxygen, and/or ozone will be augmented. For instance, the hydrolysis of polyethylene terephthalate proceeds rapidly in water at 100°C but negligibly at 70°C (Brown and Reinhart, 1971). Gutfreund (1971) has studied the thermal degradation of polyethylene but under pyrolytic conditions relating to waste management rather than environmental degradation. Studies on the potential effects of cryogenic exposure have not been encountered.

The effects of water on polymer degradation are often difficult to distinguish from the effects of fungi (Brown, 1946; Leutritz and Hermann, 1946). However, a variety of structural groups including acetals, amides, esters, nitriles, and some ketones are subject to hydrolysis. Polymers containing such groups as part of the chain backbone - e.g. polyamides, polyesters, and the regenerated cellulose fibers - will undergo chain cleavage on exposure to water (Lightbody et al., 1954). While most often referred to as an adverse reaction, hydrolysis characteristics have recently been used in the design of polymers intended to degrade in exposure to water, thus facilitating solid waste management (Anon., 1971b; Anon., 1974).

Oxygen is a major cause of polymer degradation under normal environmental exposure. Lightbody and coworkers (1954) have cited oxygen as the degrading agent of greatest economic importance causing embrittlement, cracking, and granulation in commercially important polymers such as polyethylene, nylon, and the cellulose esters. Similarly, oxygen may lead to cracking, embrittlement, or tackiness in some synthetic rubbers. On the molecular level, oxygen attack may result in crosslinking, chain cleavage, oxygen addition, or hydroperoxide formation, and often involves autoaccelerating free radical reactions (Gutfreund, 1971; Scott, 1970).

Ozone is involved in similar types of polymer degradation reactions initially involving chemisorption (Gutfreund, 1971). Particularly affected by ozone are polymers containing a carbon-carbon double bond. Thus, most of the commercially important synthetic polymers are not especially attacked. Even neoprene rubber  $([-CH_2-\overset{C\ell}{\overset{|}{C}} = CH-CH_2-]_x[-SS-]_y)$ is not extremely sensitive to ozone because the double bond is deactivated by the adjacent chlorine (Lightbody et al., 1954).

Perhaps the key factor in the non-biological degradation of synthetic polymers is light. In terms of the engineering of degradable polymers, photodegradation seems to be the most productive approach (Titus, 1973). A variety of photosensitizers, such as benzoin, cobalt nitrate, manganese nitrate, and cobalt chromate, may be added to polymer formulations to increase the rate of photodegradation (Gutfreund, 1971). However, many polymers are inherently photodegradable. A photodegradable 1,2- polybutadiene resin has been developed in which the rate of degradation is directly proportional to the crystalinity (Anon., 1971a). Similarly, a polybutene-1 film, reportedly degrades into inert hydrocarbon powder after exposure to light for varying periods (Anon., 1972). In addition to polybutene and polybutadiene, many commercially important polymers - such as polyethylene, polystyrene, polypropylene, acrylonitrile-butadiene-styrene, and polyvinyl chloride - undergo photolytic reactions (Titus, 1973).

The importance of structural impurities in the physiochemical polymers has been repeatedly emphasized (e.g. Searle, 1971). For instance, polypropylene,  $CH_3-(CH_2-\overset{CH_3}{\overset{|}{C}H}-)_nCH_3$, if pure, would be transparent, would not absorb light, and therefore would not undergo photodegradation. However, polypropylene contains ketone groups which absorb ultraviolet irradiation and result in Norrish Type I chain scission or Norrish Type II hydrogen extraction (Cooney *et al.*, 1973). Similarly, ketone groups have been purposely added to polystyrene to accelerate photodegradation (Titus, 1973). Impurities are conducive to other types of physiochemical attacks besides photolysis. Polyethylene $CH_3-(CH_2-CH_2)_n-CH_3$, for instance, contains unsaturated carbon-carbon bonds which can be attacked by oxygen or ozone and result in either direct chain cleavage or hydroperoxide formation (Gutfreund, 1971).

As indicated previously, the physiochemical agents which can degrade polymers seldom act in isolation from one another. This is illustrated in Figure 54 with the mechanism for the degradation of polyethylene in which the effects of impurities are discounted (Gutfreund, 1971).

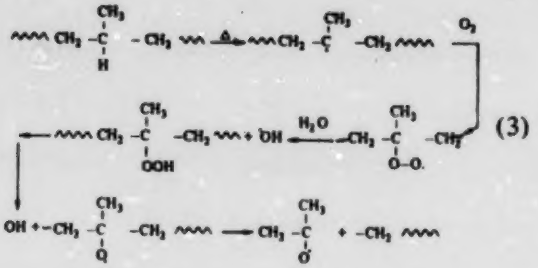


Figure 54. Physiochemical Degradation of Polyethylene (Gutfreund, 1971)

Although considerable progress is currently being made in development of degradable polymers and the elucidation of the structural components which are conducive to degradation, structure-degradability relationships, other than the rather general characteristics outlined above, cannot be drawn. However, two points deserve particular emphasis. First, the physiochemical degradation of polymers does not usually result in monomeric units but rather ends in a wide range of intermediate size molecules. This has been illustrated in polyethylene (Gutfreund, 1971) and also seems to apply to polybutene-1 (Anon., 1972). Secondly, physiochemical degradation does not necessarily lead to biological degradation. This has been shown in the photodegradation of polypropylene (Cooney, et al., 1973). Thus, while an ideal polymer might be one that physiochemically degrades into units which can be biodegraded to carbon dioxide and water, this pattern has not been demonstrated in polymers which have been termed "degradable".

IX. CATEGORIZATION OF CHEMICALS IN TERMS OF THE SUITABILITY OF VARIOUS TEST METHODS

After the possible persistence and degradation processes have been theoretically considered (see Section VIII, p 461), an appropriate experimental procedure needs to be developed to test the chemical's environmental behavior. Deciding on the appropriate procedure requires the consideration of the physiochemical properties, quantities and sources of release to the environment (release potential), commercial-economic factors of the compound of interest, and the toxicity.

There are well over 2 million chemical compounds registered with the American Chemical Society's Chemical Abstract Service (Council on Environmental Quality, 1971). Fortunately, only 9 - 10,000 synthetic organic compounds are in commercial use. These commercial (or potentially commercial) compounds (and appropriate model compounds, to establish meaningful theoretical models and generalizations) are the ones that should be tested since materials produced in large quantities have a high potential for becoming environmental contaminants. However, compounds likely to pose hazards because of their potential toxicity should be tested too, even if their production is small.

Setting priorities for research on these thousands of commercial compounds is an extremely complex process requiring the consideration of environmental release potential, environmental stability, and toxicity (Howard, 1974). Some of these same factors can be used for deciding which test method should be used. For example, the quantity produced, the frequency of release, the environments through which the chemical passes, and toxicity should have a considerable effect on the technique chosen. The quantity, toxicity and potential for release to the environment of the chemical provides an approximate indication of the degree and complexity of the testing required. Thus, for compounds in the developmental stage of commercialization, probably only a screening test is justified. As commercialization continues, more detailed studies should be undertaken. When a compound is found to be produced and released into the environment in large quantities or in concentrations that might be toxic or subject to biomagnification, it should receive an indepth (and extensive) study. Of the three parts of a test system (chemical, analytical methods, test media), the analytical methods provide the widest spectrum of desirable information and costs. The indirect methods, such as oxygen uptake, carbon dioxide evolution, and ultraviolet spectroscopy, are relatively inexpensive but provide no insight into the degradation pathways. In contrast, using radioassay techniques, although expensive, allows a mass balance of the breakdown products and considerable insight into the degradation that may take place in the environment.

Although chemicals in our environment often move from one medium to another, they frequently are first released into one specific medium. For example, volatile materials usually vaporize into the atmosphere, (vapor pressure important), detergents usually pass through sewage treatment plants or a septic tank, and plastics usually end up buried in landfills. Therefore, although a chemical that enters the environment should be tested for persistence in a variety of media, some priorities can be provided by the routes into the environment and likely points of residence in the environment. Thus volatile materials ought to be tested in atmospheric chambers (e.g. smog chambers, LP1R, plastic or glass containers). Hobbs (1974) has concluded that chemical species with molecular weights substantially higher than 200 to 300 will not reside in the atmosphere as molecular species (vapors). Non-volatile, water-soluble materials should be tested in aqueous media and the media in which the material enters the environment, but not in atmospheric systems. A contaminant found in water effluents should be tested under sewage treatment and natural water conditions. The time of the year that the chemicals are used can also be important. For example, Evans et al. (1973) studied urea under winter conditions because of its heavy use during that season (de-icing agent).

The frequency of environmental discharge may sometimes be an important factor in determining the degree of acclimation to be allowed. Buzzell et al. (1969) found that degradation of compounds which were slug loaded into activated sludge treatment plants was best modeled by using unacclimated sludge. Swisher (1970) concluded that with surfactants which are usually fairly evenly loaded into sewage treatment plants, acclimation should be allowed to develop to its fullest. Knowing the degradation rate under both acclimated and unacclimated conditions can sometimes provide considerable insight into biodegradability.

Physical and chemical properties of the compound, besides being indicative of routes into the environment and mobility from one medium to another, may also have a considerable impact on the technique to be used. For example, volatility (vapor pressure) may be an important consideration in design of the experimental apparatus. In atmospheric studies, some low vapor pressure materials have a tendency to enter into unrecognized wall reactions and are difficult to inject into the reaction vessel. Glasson and Tuesday (1971) used a heated manifold in order to expand low vapor pressure compounds into their long-path cell. Stephens (1973) used a stirred flow procedure with glass carboys to study aromatic compounds with minimum loss of reactants due to adsorption on the walls. Crosby and Moilanen (1974) used a special apparatus which compensated for wall reactions for studying the vapor phase photolysis of pesticides.

In contrast, highly volatile compounds require special precautions in soil and water systems. For closed systems, such as BOD, volatile compounds cause little difficulty. However, where the reaction vessel is open to the atmosphere, physical loss can result. Several approaches to compensate or control such losses have been used. Metcalf and Lu (1973) used a system with a vapor trap instead of an open aquarium when working with relatively volatile materials. When working with laboratory pilot plant activated sludge treatment plants, Buzzell _et al_. (1969) ran stripping studies to determine volatilization losses.

Another physical property that determines the suitability of the experimental procedure and method is the water solubility of a chemical; most water insoluble (hydrophobic) compounds tend to adsorb on particulate matter and other solid surfaces. This effect is especially prominent when testing for persistence in an aqueous system. If the compound is water soluble, aliquots of the aqueous solution can be sampled to determine degradation. However, when the compound is very water insoluble, (e.g., organochlorine pesticides) a number of identical samples are prepared so that the whole sample can be extracted. For example, when using the river die-away test with a number of pesticides, Eichelberger and Lichtenberg (1971) prepared fifteen dosed samples,and a complete sample was extracted at varying times up to eight weeks. A similar procedure was reported by Halvorson _et al_. (1971) using the shake culture test with a number of insecticides, and by Leigh (1969) using a modified Bunch and Chambers (1967) test with chlorinated hydrocarbon insecticides. Water solubility may also be important in soil systems where adsorption on soil particles may reduce the degradation rate and complicate the analytical extraction procedure. On the other hand, water soluble ionic species (e.g. diquat and paraquat, Funderburk, 1969) may also be tightly bound to soil particles. Water insoluble materials may also give misleading results in activated sludge treatment plant systems. For example, Choi et al. (1974) reported that an activated sludge system removed a high percentage of PCB's contained in waste water. However, PCB's were adsorbed on the sludge but not degraded and, therefore, the PCB would be deposited wherever the sludge was dumped (landfill, land spread, or the ocean).

In summary, commercial-economic, environmental release, biological effects, and physical-chemical properties of a chemical can be used to determine the extent and type of testing for environmental persistence and fate as well as determine the experimental procedure. Some important parameters include (1) quantity produced and released into the environment, (2) route and frequency of release into the environment, (3) volatility, (4) toxicity, and (5) water solubility.

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